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SULFURIC ACID TREATMENT OF COTTON SEED

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In recent years, the sulfuric acid treatment of cotton seed has been advocated as a preventive measure for certain diseases attacking cotton, notably the bacterial disease known as Angular Leaf Spot, Black Arm, or Boll Rot, and the fungal disease known as Anthracnose.

The results obtained by the American workers Rolfs, Faulwetter, Ludwig and others justified an experimental trial being carried out on a small scale in the Sudan, more especially in relation to the control of Black Arm disease of cotton.

An initial series of experiments was carried out to note the effect of acid treatment on the germination of the seed. The seed used was Egyptian Sakel from the previous cotton crop, and had been ginned six months previously. The seed was soaked, for varying periods, in an enamel tin with different dilutions of sulfuric acid, then washed in water, dried, and sown two to three inches deep in moist soil contained in pots—20 seeds to each pot.

It is unnecessary to give the details of these experiments; suffice it to say that 500 gm. of cotton seed could be treated with 100 cc. concentrated sulfuric acid for 1 hour, washed in 2 liters of water for 10 minutes, either sown at once, or dried and then sown, with a result of 95 per cent germination. Seed in such a bulk was intimately brought in contact with the acid by mixing with a wooden spoon, and completely delinted in a short time. The final result showed slight charring of the seed coat to a black color, and in some instances, the seed was completely decapsulated without any apparent adverse effect on germination.

This experiment was modified by washing in water for longer periods, and it was found that 1 hour was the maximum period for washing without affecting germination. Longer periods of washing tended to swell the seed coat, and gave a lower percentage of germination.

Seed treated with concentrated sulfuric acid in the above proportions, washed for 10 minutes, dried, and stored for 6 months, showed, after sowing, 92 per cent of germination, whereas, seed similarly treated and washed for one hour in 2 liters of water, dried, and stored for six months gave 87 per cent germination.

[1] Director.

The acid treatment of seed whether concentrated or in dilutions of 1/20, 1/100, 1/200, 1/500 for periods longer than 1 hour affected adversely the germination of the seed. In periods of treatment less than, and up to, 1 hour, seedlings usually appeared above the soil in five days.

Comparative field observations were carried out, on a large scale, with seed treated in the proportion of 500 gm. of seed to 100 cc. concentrated sulfuric acid for fifteen minutes to half an hour, washed, dried, and sown two months later, and with seed untreated and sown under the same conditions. The treated seed showed better germination than the untreated, and resulted in a healthier plant with better vegetative growth. Data regarding the final yield of cotton are being collected.

SULFURIC ACID TREATMENT AS A PREVENTIVE MEASURE FOR BLACK ARM

It may be stated at the outset that sulfuric acid treatment of Black Arm infected cotton seed has not given the results claimed by American workers. Preliminary pot experiments were carried out; cotton seed from an infected source being treated with sulfuric acid and sown under conditions which excluded the possibility of infection from soil, water, or insects. Seed treated with concentrated sulfuric acid for 1 hour and 5 minutes yielded, in one experiment among others, 5 per cent of seedlings with typical angular leaf spot infection of the cotyledons.

Investigations by the writer have shown that the causal organism of Black Arm can be recovered and grown from the tissues internal to the seed coat; short of destroying the germination of the seed, it is difficult to conceive of acid treatment affecting the vitality of bacilli within the resin canals and tissues of the embryo. Organisms lying immediately below the seed coat could certainly be adversely affected by acid treatment. It would appear then, that at the most, a partial sterilization of the seed can result from sulfuric acid treatment.

Field observations by the writer show that there can be no doubt, however, that acid treatment exerts a beneficial effect, by delaying the manifestations of Black Arm disease and so by permitting the plant to obtain a good stand before infection is apparent. More information, however, is required regarding the predisposing factors concerned with the spread of the disease. Environment, climate, soil, and farming methods appear to play a major or minor part, and are being investigated.

SUMMARY

1. Experiments were carried out to test the effect of sulfuric acid treatment on the germination of cotton seed.

2. Seed treated in the proportion of 500 gm. to 100 cc. concentrated sulfuric acid, washed for 10 minutes in 2 liters of water, dried, and then sown yielded 95 per cent germination.

Seed treated in such a manner and stored for 6 months gave 92 per cent germination.

3. Seed treated with concentrated sulfuric acid for longer periods than one hour, or washed for longer periods than one hour after treatment was adversely affected as regards germination.

4. Field observations on treated and untreated seed showed better germination and plant growth in favour of the former.

5. Sulfuric acid treatment will not completely sterilize Black Arm infected seed but appears beneficial because it delays the manifestations of the disease in the cotton plant.

BLACK ARM DISEASE OF COTTON WITH SPECIAL REFERENCE TO THE EXISTENCE OF THE CAUSAL ORGANISM B. MALVACEARUM WITHIN THE SEED

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Wellcome Tropical Research Laboratories, Khartum

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In certain countries, where cotton is grown on a large scale, considerable losses in yield result from the ravages of a disease known as Angular Leaf Spot of Cotton, Black Arm, and Boll Rot, names which merely indicate different manifestations of the same disease and which may all occur at the same time in the same plant.

The causal organism *B. malvacearum* was first isolated by Smith (6), who proved its pathogenicity by infection experiments, and since then its causal relationship to the disease has been fully established by other workers.

As to the method of infection of the plant, conflicting views have been held by different observers. Hibbard (4) in 1910, and Jenkins (5) somewhat later, mention that infection may be carried in the seed, and Faulwetter (2) considers that seed may be a probable factor in primary infection of the plant but considers that wind-blown rain plays a more important part in disseminating the disease throughout a cotton crop. In America, the results of seed treatment by means of chemicals supported the view that the seed coat harbored the causal organism, but the records (1, 3) show that treatment, even with concentrated sulfuric acid, failed to sterilize completely infected seed.

As far as the writer is aware, no serious attempts have been made by investigators to prove whether infection was on the seed coat and its appendages, lint and fuzz, or whether the infection was within the seed, indeed, from the literature available, it appears to have been generally accepted that the infection was on the seed coat with its attached lint and fuzz.

As the question was of importance, especially from the point of view of seed treatment on a large scale, the writer, twelve months ago, carried out some investigations. Some preliminary experiments with cultures of *B. malvacearum* showed that the organism was endowed with feeble resisting qualities against sunlight, heat, and desiccation and all attempts to demonstrate the existence of spores in cultures proved negative.

With such facts, which confirmed the work of other observers, it was difficult to support the hypothesis that infection was maintained on the outer coat of

[1] Director.

the seed, and indeed, a large number of cultural experiments subsequently carried out with the lint, fuzz, and the outer coat of the seed, failed to show the presence of *B. malvacearum*.

A further experiment served to advance the hypothesis that infection was internal to the seed coat; seeds from infected plants were placed in a hot-air oven, and heated to a temperature of 70°C. for 1 hour, a temperature well beyond the thermal death point of the bacillus. The seeds were removed and placed in sterilized soil (which had previously been sterilized by heat at a temperature of 180°C.) in sterile pots, the soil watered daily with sterile distilled water, and the pots placed under conditions free from insect attack. Germination occurred and the young cotyledons soon showed a heavy infection with angular leaf spot, the bacillus being readily recovered from the lesions.

Attempts were then made to recover the bacillus from the tissues within the seed coat.

Several techniques were employed and abandoned, the difficulty being in sterilizing completely the outer coat of the seed without affecting the interior. The use of alcohol, however, surmounted this difficulty.

Ginned cotton seeds, i.e. seeds which had been collected from cotton plants attacked with black arm twelve months previously, were placed in absolute alcohol for 3 minutes, removed with a pair of sterile forceps, and ignited in a flame in order to sterilize the outer coat of the seed. When the alcohol was completely burnt off, the seeds were then dropped into tubes of sterile distilled water and incubated at a temperature of 31 to 33°C. for 48 hours; by this time the seed coat had considerably softened, and in many instances had split. A sterile glass rod was then inserted into the tubes containing the seeds and distilled water, and the seeds were thoroughly crushed up. The tubes were then incubated for 24 to 36 hours at 31°C. and subcultures prepared on agar slopes.

In some instances, a pure culture of *B. malvacearum* was obtained in 20 per cent of seed taken from infected plants, the presence of *B. malvacearum* obtained from seed by this method was always verified by a study of the cultural and other characters of the organism, by its biochemical reactions, and finally by inoculation experiments on cotton seedlings in order to test its pathogenicity.

Having proved that infection was within the seed coat, attempts were made to section the seed to demonstrate the site of the organism contained therein.

Preliminary treatment of the seed with nitric acid was necessary to soften the coat, followed by fixation in graded alcohols, by clearing, and by embedding in paraffin. Sections were made and stained by Gram's method, thionin blue, and haematoxylin. Examination showed the organisms occupying the resin canals (plate 1, fig. 1) and the cotyledonous tissue (plate 1, fig. 2).

The same technique was employed in recovering the causal bacillus from the plant tissues below the site of lesions. Cotyledon leaves showing typical

angular leaf spot were removed and their supporting stems examined with a lens for evidence of gross lesions. Stems, free from lesions, were soaked in absolute alcohol, ignited, and then crushed in sterile distilled water, and agar cultures prepared. By this somewhat rigorous technique, it was possible to obtain *B. malvacearum* in the tissues and sap of apparently healthy areas below infected lesions; this was applied with similar results to tissues of the main stems supporting infected bolls.

It would appear then from these laboratory experiments, that infected seed was capable of producing infected plants and that the bacillus could be isolated from apparently uninfected tissues below black arm lesions, indicating possible latency on the part of the organism—a point which appears to be supported also by field observations.

The other likely sources of infection to the plant may be considered under the following headings: (*a*) soil, (*b*) water, (*c*) insects, and (*d*) ginning factories. As regards soil, the writer has carried out a large number of examinations of samples, taken at various depths, of cultivated and fallow soil, and has, so far, failed to recover *B. malvacearum;* the same remarks hold good for water samples taken from irrigation canals, and collections of standing water in cotton areas attacked with black arm. The feeble powers of resistance of the organism to such adverse conditions as sunlight, desiccation, and high temperature, as hold in the Sudan, make it difficult to accept any hypothesis indicating the soil as a reservoir of the virus. As regards the disease being insect borne, no epidemiological evidence exists in the Sudan to support such a view. In the early stages of the cotton plant, the flea beetle is the predominant pest, its ravages are obvious, yet plants free from attack show evidence of disease, and plants attacked show no signs. The same remarks apply equally to such cotton pests as thrips, aphis, stemborer, and white ants. Moreover, laboratory examinations of these insects have so far failed to incriminate any of them as vectors of the causal organism of black arm. The possibility of infection being maintained in ginning factories has been considered, a hypothesis which may be dismissed in view of the delicate nature of the organism concerned.

Faulwetter's theory that the disease is disseminated by the combined action of wind and rain is not supported by the extensive and careful field observations carried out by Bedford.[2]

No other plant hosts have been found either manifesting the disease, or harboring the bacillus; indeed, the writer has failed so far to reproduce lesions by culture inoculation into plants other than cotton. It would appear, therefore, that *B. malvacearum* is specific for the cotton plant.

The factors predisposing to the manifestation of the disease—soil, climate, and imperfect agricultural methods—are at present being investigated. The problem is beset with difficulties in view of what has been stated regarding

[2] Gezira Entomologist.

the possible latency of manifestations of infection. In this connection, investigations are now proceeding to ascertain whether healthy plants harbor the virus without showing evidence of infection. Field observations have shown that certain varieties of American rain grown cotton are more resistant to attack than others, a resistance shared also by rogue cotton and some ratoon cotton. It may be that such resistance is dependent on the pH reaction of the sap, a hypothesis worthy of investigation.

Seed treatment, by means of sulfuric acid, has been advocated as a remedial measure for the disease but, in the writer's experience, has not proved the success claimed by American workers. In view of the writer's investigations regarding infection within the seed coat, it is difficult to conceive any form of seed treatment, short of destroying the germination power of the seed, effectually sterilizing infected seed. Sulfuric acid treatment appears beneficial to the germinating properties of the seed, and assists by producing a healthier plant in its early stages, but experience has shown that such a plant, in its later stages of growth, often shows manifestations of the disease. The writer is of the opinion that more information regarding the predisposing factors of the disease is required, and when this knowledge is acquired, it will be possible to grow and maintain a healthy type of plant capable of resisting, or throwing off, infection when once attacked.

SUMMARY

1. Investigations have shown that, in Black Arm disease of cotton, the causal organism can be recovered from the tissues within the seed coat.

2. A technique is described for recovering the bacillus from the seed tissues.

3. The feeble resisting powers of the organism toward such adverse conditions as strong sunlight, desiccation, and high temperature render it unlikely that the outer coat of the seed, with its lint and fuzz, harbors infection.

4. The bacillus can be recovered from apparently healthy tissues below black arm lesions.

5. The seed appears to be the main source of infection.

6. The causal organism has not been found in soil or water, and the epidemiology of the disease does not favor the hypothesis that the disease is insect borne.

7. No hosts other than the cotton plant have been found.

8. Seed sterilization by means of concentrated sulfuric acid has yielded disappointing results. A more effectual way of attacking the problem is to ascertain the factors that predispose to manifestations of infection, and to raise a healthy type of plant capable of resisting as well as of throwing off infection when attacked.

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PLATE 1

FIG. 1. Transverse section of Black Arm infected cotton seed showing bacilli in a resin canal. × 960.

FIG. 2. Section of Black Arm infected cotton seed with bacilli in the cotyledon tissues. × 800.

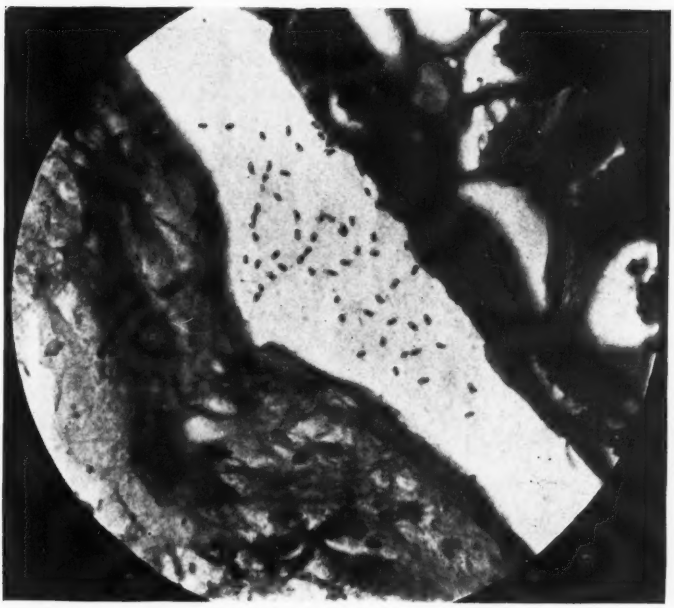

FIG. 1

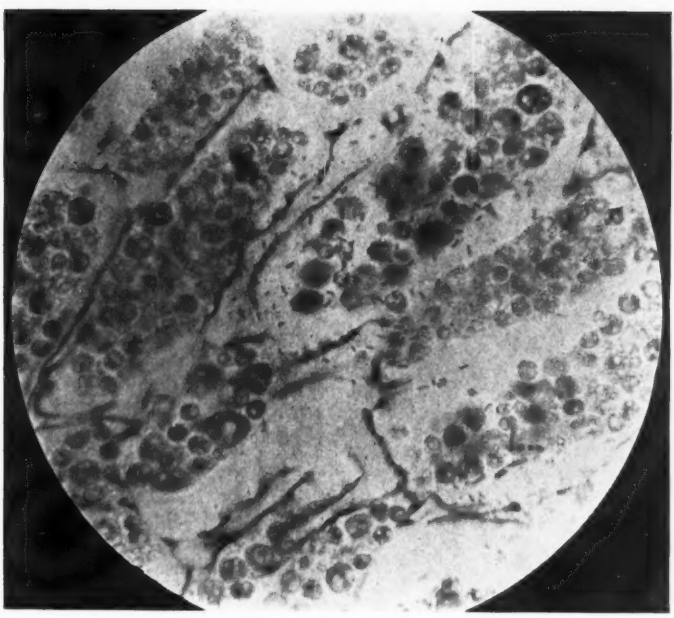

FIG. 2

THE USE OF COLLODION SACKS IN OBTAINING CLEAR SOIL EXTRACTS FOR THE DETERMINATION OF THE WATER-SOLUBLE CONSTITUENTS[1]

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INTRODUCTION

A clear extract, essential in the analysis of the water-soluble constituents of the soil, is especially important where colorimetric methods are to be used. Difficulties in obtaining soil extracts unaltered in composition and free of all colloidal material, emphasize the desirability of a simple method that could be used in obtaining clear extracts for the determination of the water-soluble constituents of soils.

The methods used in obtaining soil extracts are: centrifuging or ordinary filtering with or without the use of flocculating agents, filtration by suction, filtering through Pasteur-Chamberland filters, and dialysis through collodion sacks.

It is generally recognized that ordinary filtering and centrifuging of soil suspensions do not give clear extracts with many soils. In order to overcome this difficulty various agents have been used to flocculate the colloidal particles that usually pass through the filter paper or remain in suspension. Although flocculents have proved very successful, they cannot be used in obtaining soil extracts for the colorimetric determination of H-ion concentration, nor for a determination of the basic constituents of the soil. In the latter case there is always the possibility of solubility effects as a result of base exchange.

The suction filtering method is based on the principle of untrafiltration through a thin layer of soil deposited on the filter paper, and, as recently used by one of the authors (21), gives a clear extract very readily. This method, although simple, requires a little care in manipulation, and is rather time consuming where a large number of extracts is to be obtained.

The Pasteur-Chamberland filter method gives large amounts of clear extract, but requires rather expensive equipment. Moreover, it cannot be used in H-ion work nor, as was recently shown by Parker (19), where phosphorus is to be determined in the extract. In order to study further the influence of the Pasteur-Chamberland filter on the composition of the extract the following

[1] Published with the permission of the Director of the Alabama Agricultural Experiment Station.

experiment, the results of which are given in table 1, was conducted. Extracts were prepared by dialysis from two soils which gave extracts of widely different composition with respect to several ions. The extracts were passed through thoroughly washed filters and the change in composition was determined. The filters were then washed with 300 cc. of distilled water, and a portion of the original extract from the other soil was passed through the filters. For example, a non-acid extract rather high in phosphorus was filtered through filters 5 and 6. Then these two filters were washed and used with an acid soil extract, low in phosphorus. In this manner the influence of filters used with one extract upon another extract subsequently filtered was determined.

TABLE 1

The composition of the soil extracts as influenced by Pasteur-Chamberland filters

SOIL NUMBER	SOIL	TREATMENT OF DIALYZED EXTRACT	SPECIFIC RESISTANCE	H-ION CONCENTRATION	PHOSPHORUS	CALCIUM	POTASSIUM	NITRATE NITROGEN
			ohms	*pH*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
27	Silt loam	None	10,475	6.60	1.55	11.6	3.4	3.7
		Passed through filters 5 and 6*	11,420	6.65	1.25	11.1	5.4	3.7
		Passed through filters 7 and 8†	11,700	6.62	1.25	9.7	5.5	3.7
28	Silt loam	None	14,810	4.70	0.05	4.7	6.6	3.1
		Passed through filters 7 and 8*	14,615	5.73	0.04	5.6	6.4	3.1
		Passed through filters 5 and 6†	14,810	5.80	0.12	5.5	7.5	3.1

* Filters were washed with 800 cc. distilled water before filtering the extract.

† Filters were those used under * for the other soil but were first washed by passing 300 cc. of distilled water through them.

It will be seen from table 1 that the filters had a marked effect on the phosphorus and H-ion concentration of the extracts. Thus, the H-ion concentration of soil 28 was changed from pH 4.7 to pH 5.7 by filtering. The phosphorus content of the extract was little affected with one filter whereas with the filter that had previously been used with a soil high in phosphorus, the phosphorus content of the extract was more than doubled as a result of passing through the filter. In soil 27, the filters absorbed about 32 per cent of the phosphorus in the extract, whereas the H-ion concentration was not affected. The calcium and potassium contents of the extract were affected to a less extent, although the data indicate that the filters may either retain or give up appreciable amounts of calcium and potassium. The nitrate content, as would be expected, is not affected by filtering.

The collodion sack method of obtaining extracts has been used in biological studies for a long time, but it has not been used extensively in soil investiga-

tions. Knight (11), Mirasol (16), Denison (5), and Magistad (15) used collodion sacks in a study of the nature of the aluminum compounds in the soil extract. As far as the authors know, Parker and Tidmore (20), working in this laboratory, were the first to use the method for obtaining soil extracts. They made extensive use of it in their studies on the phosphorus content of the soil solution and soil extracts. Their method, in brief, consisted in placing the collodion sack containing the soil suspension in a covered beaker containing a definite amount of water for eighteen to twenty hours to allow equilibrium to become established between the inside and outside solutions. The contents of the sack and beaker were shaken occasionally.

Because of the apparent possibilities of this method in overcoming some of the objections of the common methods of obtaining soil extracts, this study was undertaken. This paper presents the results of a study made with regard to:

(*a*) The method of preparing sacks and extracts, (*b*) the time necessary for the establishment of equilibrium between the solution inside and outside of the sacks, and (*c*) the use of the method in determinations of the H-ion concentration, nitrate nitrogen, phosphorus, and calcium content of soil extracts.

METHODS

The collodion sack method of obtaining a soil extract is based on the principle of dialysis or diffusion of soluble material through a collodion membrane. The soil suspension is placed in a collodion sack, which is then placed in water, and equilibrium is allowed to become established between the inside and outside solutions. After equilibrium is established, the inside and outside solutions are apparently of the same concentration with respect to all ions determined in soil extracts. Apparently there is no Donnan equilibrium established to cause a difference in concentration of any ion in the inside and outside solutions. This may be explained by assuming that there are no non-diffusible ions in the soil suspension. Subsequent data will show that the concentration of all ions studied was the same in the inside and outside solutions after equilibrium was established.

Preparation of sacks

In the preparation of the collodion sacks Loeb's (14, p. 109) procedure was used with some minor modifications. The sacks were made in flasks, the size depending on the size of sack desired or the amount of suspension to be dialyzed. Thus, where only about 50 cc. of soil extract was desired, as for the determination of nitrates and of H-ion concentration by colorimetric methods, 150-cc. extraction flasks were found very satisfactory. These flasks give sacks with wide necks through which the soil can be readily poured. When more extract was needed, sacks made in 500-cc. Erlenmeyer flasks were used. The procedure for making the sacks is as follows:

Merck's Blue Label collodion is poured into a thoroughly cleaned flask, which has been rinsed with 95 per cent alcohol to remove all water. It is essential that the collodion solu-

tion be kept free of water, for sacks made from a solution containing even small amounts of water are opaque and weak. While rotating the flask slowly by hand the collodion is poured back into the bottle during a period of 2 minutes. The flask now has a thin, uniform layer of collodion on the inside, which is allowed to dry by placing the flask in an inverted position in a ring stand for 8 to 10 minutes. It is then placed under a faucet and washed by allowing water to run into it 3 to 5 minutes. The water is then poured out, the edges of the neck of the collodion membrane are loosened a little from the neck of the flask, and water from the tap is allowed to run between the sack and flask. This procedure frees the sack from the walls of the flask, and the sack can then be readily removed and placed into a jar of water until used. By adding a little toluene to the water, mold growth is prevented and the sacks may be kept practically indefinitely.

Where a number of sacks are to be made, the authors work with six flasks. While the membrane is being formed in one flask, three flasks are drying and two are being washed. Using this system one person can make 15 to 20 sacks an hour.

The viscosity of the unused collodion gradually increases because of the evaporation of the solvent. As a result the sacks made from this solution become too thick. When this condition is noticed, ether must be added to the solution. The amount of ether needed can be readily estimated after a little experience. By the addition of ether as it becomes necessary, 75 to 90 sacks (made in a 150-cc. extraction flask) can be made from one pound of collodion.

Methods of controlling the permeability of the collodion membranes have been the subject of several investigations. It was first pointed out by Bigelow and Gemerling (1) that the longer collodion membranes are allowed to dry, the less permeable they become. Brown (2) found that if dry sacks are placed in different strength solutions of alcohol, those in the more concentrated alcohol solutions become more permeable and fragile than sacks placed in weaker solutions of alcohol. Schoep (22) found that sacks are more permeable if a little oil or glycerine is added to the collodion from which the sacks are made. Among others who worked on the permeability of collodion membranes are: Nelson and Morgan (17), Browne, R. J. (3), Eggerth (6), and Farmer (8).

Most of these workers, however, were working with membranes to be used for ultrafiltration. For dialysis work, as used ordinarily, it seems that the simple procedure used by Loeb in all his work and outlined above gives sacks of good permeability along with a fair degree of strength. In some preliminary work the writers found that the permeability of the sacks was practically the same whether they were dried two or thirty minutes before washing.

Procedure used for obtaining the soil extracts or dialysate

In the preparation of the soil extracts the sacks were removed from the jar, rinsed in distilled water and placed in the same kind and size of flasks as those in which they were made. The soil was then poured into the sacks by means of a short, wide-stem "powder-funnel" and the required amount of water added partly to the inside and partly to the outside of the sack. Where the extracts were desired for the determination of nitrates and of H-ion concentration only, 1:2 extracts were used, one-half of the water being added to the inside of the sacks. For the determination of all other ions where large amounts of extract were desired, 1:5 soil-water extracts were obtained. In that case only one-fourth of the water was added to the inside, the remaining water being added to the outside of the

sack. Thus large amounts of extracts were obtained. The necks of the sacks were then folded against the necks of the flasks, clean rubber stoppers inserted, and the flasks shaken. The shaking was done by hand and was repeated every hour during the day. After a period of eighteen hours the dialysates were removed from the sacks and used for the various analyses.

The use of flasks closed with rubber stoppers instead of beakers is not only highly desirable if the H-ion determination of the dialysates is to be determined, but it facilitates shaking and also makes possible the using of a mechanical shaker for the continuous shaking of the system. As subsequent data will show, the shaking of the flasks by means of a drawer type mechanical shaker causes the establishment of an equilibrium between soil and water in a shorter period of time. If hand shaking is to be used, however, covered beakers can be used as well as flasks. With the use of beakers it was found convenient to place water on the inside and the soil suspension on the outside of the sacks.

The sacks are easily cleaned and can be kept in a jar of water until again used. It was found that a sack may be used six to ten times, provided it is not allowed to dry.

Time required for the establishment of equilibrium

The time required for the suspension and dialysate to come to equilibrium depends largely on two factors; namely, the kind of ion being considered, and the kind and amount of shaking to which the system is subjected. Different ions have different diffusion velocities and they also come to equilibrium with the soil at different rates, because of variations in solubility. For the purpose of this study the ions are considered in two groups; first, readily soluble ions such as hydrogen, chloride, and nitrate; second, ions with which there may be considerable solubility effects, such as phosphate, calcium, and potassium. Shaking materially influences the time required for the establishment of an equilibrium and is one of the most important factors to consider in a study of the method. As a result of preliminary work it was apparent that somewhat different procedures should be used in the equilibrium studies for the two classes of ions; therefore, the two classes of ions will be considered separately.

Easily soluble constituents. As a preliminary study, three methods of shaking—hand shaking and two types of mechanical shaking—were compared with regard to their effect on the rate of establishment of equilibrium. In the hand method of shaking, the flasks were shaken for 1 minute at 15 minute intervals. Shaking by means of the Camp rotary shaker and the drawer shaker was continuous. With both the mechanical shakers the gearing was reduced to give one complete cycle per second. The nitrate content of the dialysates was determined by the phenoldisulfonic acid method, and the H-ion concentration by the colorimetric method.

It will be seen from table 2 that drawer shaking causes a more rapid diffusion of ions into the dialysate than does either of the other two methods. Thus the average nitrate content in the dialysates after one hour is 25.3 p.p.m. with

hand shaking and 31.5 p.p.m. with drawer shaking. Similarly, in a comparison of the H-ion concentration and of the specific resistance of the dialysates, the drawer type shaking causes the most rapid diffusion of ions, whereas the rotary shaking usually gives values intermediate between the two other methods. The data in this table, however, as will be shown later, do not always represent the final equilibrium of hydrogen and nitrate ions.

As a result of this preliminary study it seemed desirable to determine the time necessary for the establishment of equilibrium with the hand shaking and with the drawer shaking methods. Since rotary shaking was inferior to drawer shaking it was not used in subsequent experiments. The equilibrium of nitrate ions and of hydrogen ions between the dialysate and the suspension

TABLE 2

*H-ion concentration, specific resistance and concentration of nitrate nitrogen in dialysate after shaking the flasks for one hour by different methods**

SOIL NUMBER	SOIL	H-ION CONCENTRATION			NITRATE NITROGEN			SPECIFIC RESISTANCE		
		Method of shaking			Method of shaking			Method of shaking		
		Hand	Rotary	Drawer	Hand	Rotary	Drawer	Hand	Rotary	Drawer
		pH	*pH*	*pH*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*ohms*	*ohms*	*ohms*
1	Norfolk sandy loam	5.23	4.85	4.90	67.9	72.2	72.5	6,480	6,920	6,050
2	Sumter clay	7.23	7.58	7.63	3.8	4.1	4.6	18,010	14,430	14,235
3	Gray silt loam	4.58	4.58	4.58	7.1	6.9	8.4	25,950	26,315	21,680
4	Greenville sandy loam	5.03	5.05	5.03	26.6	36.1	41.8	17,065	15,275	12,295
5	Dekalb fine sandy loam	6.08	6.20	6.18	16.0	15.9	22.6	20,550	18,410	14,215
6	Brown silt loam	5.88	5.88	5.85	30.2	34.3	39.2	14,130	10,675	9,840
Average of all soils					25.3	28.3	31.5	17,031	15,375	13,053

* Nitrate nitrogen calculated on basis of dry soil.

inside of the collodion sack was determined after 2 and 12 hours of drawer shaking and after 4, 18, and 24 hours of hand shaking of the system. As in the previous experiment, the drawer shaking was continuous, whereas the hand shaking was at intervals of 15 minutes for the first hour and of 1 hour thereafter, except during the night (between the fourth and eighteenth hours). The soil-water ratio used was 1:2, one-half the water being added to the inside of the sack. After the various periods of time, the dialysate was removed for analysis. The soil suspension from inside the sack was filtered by means of a modified suction apparatus, consisting of a Shaw (24) filter flask and a Hirsch funnel, as devised by Truog (26). Nitrate and hydrogen ions were determined in the filtered extract and in the dialysate. The data as given in table 3 represent the average of duplicate determinations.

TABLE 3

*Nitrate nitrogen content and H-ion concentration of soil extracts after hand and drawer shaking of flasks for different periods of time**

SOIL NUMBER	SOIL	METHOD OF SHAKING	TIME OF SHAKING	NITRATE NITROGEN		H-ION CONCENTRATION	
				In dialysate	In filtered extract from inside of sack	In dialysate	In filtered extract from inside of sack
			hours	*p.p.m.*	*p.p.m.*	*pH*	*pH*
1	Norfolk sandy loam	Hand	4	67.3	74.6	5.00	4.90
			18	70.8	71.1	4.95	5.00
			24	69.4	70.5	4.95	4.85
		Drawer	2	65.7	69.1	5.00	5.00
			12	72.9	72.4	4.90	4.90
2	Sumter clay	Hand	4	5.6	5.9	7.65	7.60
			18	5.8	5.3	7.65	7.65
			24	5.3	5.2	7.65	7.70
		Drawer	2	5.1	5.6	7.60	7.65
			12	6.1	5.9	7.70	7.70
3	Gray silt loam	Hand	4	7.2	8.3	4.60	4.60
			18	8.5	8.5	4.60	4.60
			24	7.8	8.6	4.60	4.60
		Drawer	2	8.7	8.8	4.60	4.60
			12	8.5	8.2	4.55	4.60
4	Greenville sandy loam	Hand	4	45.2	49.9	5.05	5.10
			18	50.1	50.3	5.05	5.05
			24	48.3	51.1	5.10	5.10
		Drawer	2	47.0	47.3	5.05	5.05
			12	49.3	49.3	5.05	5.00
5	Dekalb fine sandy loam	Hand	4	21.4	22.6	6.45	6.45
			18	20.8	22.8	6.50	6.40
			24	22.6	21.9	6.50	6.45
		Drawer	2	23.8	23.8	6.45	6.50
			12	25.3	21.9	6.50	6.50
6	Brown silt loam	Hand	4	52.4	61.4	6.00	6.00
			18	60.4	57.2	6.05	6.00
			24	58.6	58.8	6.05	6.05
		Drawer	2	58.0	58.5	6.00	6.05
			12	59.6	60.2	6.10	6.10

TABLE 3—*Concluded*

SOIL NUMBER	SOIL	METHOD OF SHAKING	TIME OF SHAKING	NITRATE NITROGEN		H-ION CONCENTRATION	
				In dialysate	In filtered extract from inside of sack	In dialysate	In filtered extract from inside of sack
			hours	*p.p.m.*	*p.p.m.*	*pH*	*pH*
Average of all soils.....		Hand	4	33.1	40.4	5.79	5.78
			18	36.0	35.8	5.80	5.78
			24	35.3	36.0	5.80	5.79
		Drawer	2	34.7	35.5	5.78	5.81
			12	36.9	36.3	5.80	5.80

* Calculations for nitrate nitrogen made on basis of dry soil.

It will be seen that with the six soils studied a complete equilibrium of nitrate ions is usually not established during the 4-hour hand shaking period. The 18- and 24-hour hand shaking periods as well as the 2 and 12 hours of drawer shaking, however, do establish complete equilibrium. Thus the superiority of the drawer shaking method in establishing equilibrium in a shorter period of time is again demonstrated. It will also be noticed that with most soils the 24-hour hand shaking method gave dialysates of slightly lower nitrate content than did the 18-hour. This is probably due to a little denitrification, for upon repeating the 24-hour period with and without the addition of 10 drops of toluene, it was found that the average content of nitrate nitrogen where toluene was added was 1.1 p.p.m. greater than where toluene had not been added.

From the data obtained with these six soils it appears that equilibrium of hydrogen ions is established in the 4-hour hand shaking period. Other soils worked with, however, did not attain equilibrium before 8 to 12 hours standing. It seems advisable, therefore, in order to be sure that equilibrium is established, to allow the flasks to stand over night. This means under most conditions a period of about 18 hours.

A comparison of the data in tables 2 and 3 shows that the diffusion of nitrate and hydrogen ions is very rapid at first. Thus as an average of the six soils, 25.3 p.p.m. nitrate nitrogen is found after 1 hour as compared to 31.5 p.p.m. after 18 hours of hand shaking; in other words, 70 per cent of the nitrate nitrogen had diffused through the sacks at the end of 1 hour when the hand method of shaking was used. With the use of drawer shaking, 87.2 per cent of the nitrate nitrogen had diffused through the collodion sack at the end of 1 hour.

From this study it is apparent that for the determinations of ions that come into solution readily as do nitrate and hydrogen ions, and possibly for such other ions as chloride, either the 2-hour drawer shaking or the 18-hour hand shaking methods can be used. The equilibrium is probably established by

TABLE 4

*The specific resistance and the phosphorus and calcium content of soil extracts after various periods of time**

SOIL NUMBER	SOIL	TIME	PHOSPHORUS		CALCIUM		SPECIFIC RESISTANCE	
			In dialysate	In filtered extract from inside of sack	In dialysate	In filtered extract from inside of sack	In dialysate	In filtered extract from inside of sack
		hours	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*ohms*	*ohms*
7	Gray silt loam	6	7.6	14.2	63.7	93.8	11,410	7,160
		18	11.0	15.4	71.8	74.0	8,290	7,060
		30	13.9	17.7	88.2	80.0	7,650	7,500
		48	15.4	13.2	85.6	89.2	7,650	7,810
8	Gray silt loam	6	1.87	3.31	38.2	50.4	11,970	8,920
		18	2.78	2.93	47.0	47.0	10,790	9,160
		30	2.49	2.84	47.0	46.1	9,730	8,640
		48	2.99	3.09	.....	47.0	8,800	11,720
9	Norfolk sandy loam	6	0.63	0.86	68.7	77.4	6,390	5,320
		18	0.84	0.92	67.8	67.8	5,660	4,810
		30	1.24	1.44	74.8	81.7	5,320	5,220
		48	1.60	1.72	85.9	85.9	5,220	.....
2	Sumter clay	6	Trace	Trace	88.7	106.0	10,330	8,450
		18	Trace	Trace	111.0	120.5	8,880	7,960
		30	Trace	Trace	131.0	151.0	7,500	6,930
		48	Trace	Trace	145.0	139.0	6,930	6,930
6	Brown silt loam	6	0.35	0.40	67.8	113.0	8,640	5,320
		18	0.55	0.45	78.3	94.8	6,930	5,660
		30	0.45	0.40	90.4	95.6	5,770	7,770
		48	0.50	0.50	102.6	100.9	6,010	5,770
10	Norfolk sand	6	1.30	2.10	39.2	45.2	15,300	12,220
		18	1.90	2.40	38.2	44.6	14,920	13,350
		30	2.30	2.60	46.3	44.0	12,500	12,790
		48	2.75	3.00	45.2	44.0	13,060	13,060
Average of all soils....		6	1.96	3.96	61.1	81.0	10,673	7,898
		18	2.85	3.68	69.0	74.8	9,246	8,000
		30	3.39	4.16	79.6	83.1	8,078	7,808
		48	3.87	3.59	85.3	84.3	7,945	8,410

* Calculations for phosphorus and calcium made on basis of dry soil.

hand shaking in less than 18 hours, but since it seemed best to allow the flasks to stand over night, 18 hours appeared to be the most practical period of time. Thus, if the flasks are started in the afternoon, equilibrium will be established by the next morning.

Difficultly soluble constituents. With the less readily soluble constituents of the soil such as phosphorus, calcium, and potassium, the process of solution is slow. With those ions which show a considerable solubility effect, such as phosphorus, a long time is required for the establishment of equilibrium between the inside and outside solutions. In explaining this fact three factors must be considered: First, equilibrium must be established between the soil and the water inside the sack; second, equilibrium must be established between the water inside and outside of the sack; third, as diffusion takes place from the inside, the equilibrium between the soil and the water on the inside is

TABLE 5

*The specific resistance and phosphorus and calcium concentration of the dialysate after 18 hours when various proportions of water are used on inside and outside of sack**

SOIL NUMBER	SOIL	AMOUNT OF WATER		PHOSPHORUS	CALCIUM	SPECIFIC RESISTANCE
		Inside sack	Outside sack			
		cc.	*cc.*	*p.p.m.*	*p.p.m.*	*ohms*
7	Gray silt loam	200	200	11.00	71.8	8,290
		100	300	10.70	75.7	8,980
8	Gray silt loam	200	200	2.78	47.0	10,790
		100	300	2.22	39.0	10,570
9	Norfolk sandy loam	200	200	0.84	67.8	5,660
		100	300	0.72	69.6	5,770
2	Sumter clay	200	200	Trace	111.0	8,800
		100	300	Trace	113.0	8,800
6	Brown silt loam	200	200	0.55	78.3	6,930
		100	300	Trace	75.5	6,930
10	Norfolk sand	200	200	1.90	38.2	14,920
		100	300	1.50	35.3	15,300

* Calculations for phosphate and calcium made on basis of dry soil.

disturbed and as a result more material comes into solution. It is a rather complex system and a true equilibrium would be obtained only after a very long period of time. Thus, in preliminary work it was found that the amount of phosphorus and calcium in the dialysates increased slightly from the second to the third day. It would seem, therefore, that the hand method of shaking would be the most practical. To find the length of time necessary for the establishment of equilibrium of the difficulty soluble ions with the use of the hand method of shaking, the following experiment was conducted. Soil dialysates were prepared by placing 80 gm. of soil and 200 cc. of distilled water on the inside of the 500-cc. sack, and 200 cc. of water on the outside of the sack.

The shaking was done by hand as in the previous experiment with nitrate and hydrogen ions. After 4, 18, 30, and 48 hours the dialysates were removed for determinations of phosphorus, calcium, and specific resistance.

The data are reported in table 4. It will be seen that with time, there is a gradual decrease in the specific resistance and an increase in phosphorus and calcium. As is to be expected, the total diffusion of ions is large during the first six hours, and gradually decreases with time as the more difficultly soluble constituents gradually come into solution. Thus, if the average of all soils is considered it will be noticed that the increase in the amount of phosphorus in the dialysate is 45.4 per cent between the sixth and eighteenth hour, 18.9 per cent between the eighteenth and thirtieth hour, and 14.1 per cent between the thirtieth and forty-eighth hour. Similarly with calcium the increase in the second period (6 to 18 hours) is 12.0 per cent, in the third period 15.3 per cent, and in the fourth period 7.2 per cent. The same general comparison is seen in the specific resistance. It is evident, therefore, that an arbitrary time of

TABLE 6

The specific resistance, H-ion concentration and nitrate, phosphorus, and calcium content of soil extracts before and after dialyzing with new and used collodion sacks

MEMBRANE USED	EXTRACT OF SANDY LOAM SOIL					EXTRACT OF SILT LOAM SOIL				
	Specific resistance	H-ion concentration	Nitrate nitrogen	Phosphorus PO_4	Calcium Ca	Specific resistance	H-ion concentration	Nitrate nitrogen	Phosphorus PO_4	Calcium Ca
	ohms	*pH*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*ohms*	*pH*	*p.p.m.*	*p.p.m.*	*p.p.m.*
None.....	5,170	5.15	4.60	0.11	8.3	16,100	5.35	2.61	0.09	3.9
New sack.....	5,159	5.10	4.66	0.07	8.2	16,280	5.35	2.62	0.09	2.9
Used sack....	5,148	5.10	4.56	0.09	8.2	16,100	5.35	2.62	0.08	3.1

shaking and standing of the system must be chosen. From this study and from further work considered in the latter part of this paper it seems that the 18-hour period with hand shaking (20) is the most satisfactory.

Since in certain cases large amounts of extract are desired, it becomes of practical value to know whether the proportion of water on the outside of the sack could be increased, thus giving a large amount of extract for analysis. The results of such a study are shown in table 5. The data indicate that the relative amounts of water used on the inside and outside of the sack have practically no effect on the concentration of calcium and phosphorus in the dialysate nor on the specific resistance of the dialysate after the flasks have stood for 18 hours.

Does the collodion sack absorb any of the soil constituents?

The question as to whether the sacks might absorb some of the soil constituents, or cause a contamination of the extract if they are used repeatedly, was studied, and the data are reported in table 6. Two hundred twenty

cubic centimeters of soil extract was placed on the inside of the sack, and an equal amount of water placed in the 500-cc. Erlenmeyer flask. After equilibrium had been established the dialysate was analyzed for phosphorus, nitrate nitrogen, and calcium, and its specific resistance and H-ion concentration were also determined.

It will be noticed that with the exception of phosphorus, none of the soil constituents were affected even when old sacks were used. Additional experiments with soil solutions of different phosphorus concentration, showed that frequently sacks will absorb small amounts of phosphorus. The maximum error caused by this absorption was found to be approximately as follows: for solutions containing 0.4 p.p.m. PO_4, 10 per cent; for solutions containing 0.2 p.p.m. PO_4, 20 per cent; for solutions containing 0.1 p.p.m. PO_4, 25 per cent. This error is only introduced when solutions are being dialyzed. It is not a factor when soil suspensions are being dialyzed in the preparation of soil extracts. In the latter case the small amount of phosphorus absorbed by the sack is readily replaced by more phosphorus coming into solution.

COLORIMETRIC DETERMINATION OF THE H-ION CONCENTRATION

It is well established that the colorimetric method for the determination of the H-ion concentration of soils is simple, time-saving, and at the same time sufficiently accurate for most soils work. A review of the work done with the colorimetric method, and a study of the various precautions to be observed in its use were made by Pierre (21). The main precautions may be briefly summarized as follows: (*a*) Importance of obtaining clear extracts, (*b*) necessity of having neutral indicator solutions, and (*c*) need of protecting solutions from the laboratory air while centrifuging and after they are obtained. By following these precautions he was able to obtain good agreement between the colorimetric and electrometric methods of determining the H-ion concentration of soils. Good agreement has been obtained by other investigators, especially by Niklas and Hock (18).

The principle of dialysis through collodion membrane was used in H-ion studies as early as 1915 by Levy and coworkers (13) in the determination of the H-ion concentration of blood. More recently Kolthoff (12) compared the H-ion concentration of soil extracts obtained by dialysis through parchment membranes with that of soil extracts obtained by filtering. He made the determinations on the dialysate and filtered extract by the colorimetric method and also on the filtered extract by the electrometric method. His results, however, do not show a very good correlation between the three methods. This can be explained partly by the fact that soil extracts are usually too slightly buffered to be determined accurately by the electrometric method as commonly used.

In the present study the authors determined the H-ion concentration of soil extracts, obtained both by dialysis and by suction filtering, by means of the colorimetric method. The H-ion concentrations of the soil suspensions were

also determined by the electrometric method. The dialyzed extract was obtained according to the method previously recommended for nitrates and H-ion determination, that is, allowing the flasks to stand 18 hours with occasional shaking by hand.

The dialysate and filtered extract were in most cases obtained from the same flask, the soil suspension inside the sack being filtered, by the modified suction method previously described, to give the extract referred to in table 7. The electrometric determinations were made on 1:2 soil-water suspensions which had been shaken for one-half hour. A Leeds and Northrup improved type of potentiometer and sensitive enclosed lamp and scale galvanometer were used. The data obtained (table 7) show a good correlation among the three methods.

The collodion sack method has also been found very useful in determining the buffer capacity of soils. In brief the method for buffer studies consists in preparing a series of flasks, using, for example, 40 gm. of soil to 80 cc. of water. To the flasks are then added varying amounts of some acid or base, determined by the range in which the buffer capacity of the soil is desired. Barium hydroxide and hydrochloric acid were used by the writers. The flasks are then allowed to stand with occasional shaking until equilibrium is established between the soil and the acid or base added. This can be readily determined by making H-ion determinations on 5 cc. of the dialysate at intervals until there is no longer any change during a period of 24 hours. This will usually be after 48 hours. If the amount of acid or base added to the soil and the H-ion concentration of the dialysates at equilibrium are known, the buffer capacity of the soil can be readily calculated.

In a brief study of the use of parchment shells for obtaining soil dialysates for H-ion determinations it was found that with the four soils studied, new shells gave extracts of a H-ion concentration equal to that of extracts obtained by the use of the collodion sack. It was found, however, that the shells may absorb bases and acids from the soils and that these bases and acids cannot be washed out readily. Consequently, if shells were used more than once with soils of quite different H-ion concentration they were found to give up some of the bases or acids previously absorbed and to give dialysates of high or low H-ion concentration. On the other hand, collodion membranes used repeatedly with soils of different reactions showed no absorption effects and gave extracts of the same H-ion concentration as those obtained with new sacks.

The collodion sack method, therefore, is of special value in obtaining soil extracts for the determination of the H-ion concentration of soils by the colorimetric method. Although the suction filtering method makes possible the securing of extracts in a shorter time, the collodion sack method is much more rapid if a large number of extracts are to be obtained. Thus, if twenty soil extracts are to be prepared, the flasks with sacks can be set up in a period of about 20 minutes, shaken a few times that afternoon, and the H-ion determinations made by the colorimetric method on the dialysate the next

morning in less than half an hour. Moreover, the collodion sack method offers fewer difficulties in the securing of clear soil extracts than does the suction filtering method. As was shown by one of the authors (21) the latter method requires that care be taken in washing the filter paper, and in preventing too much suction when soils of low H-ion concentration are filtered.

DETERMINATION OF NITRATES

The phenoldisulfonic acid method is commonly used for the determination of nitrate in soils. It is simple, rapid, and sufficiently accurate for all practical purposes, provided a few precautions are followed. Probably the most important of these precautions is the securing of a clear and colorless extract. In order to get clear extracts various methods of obtaining soil extracts have

TABLE 7

The H-ion concentration of soil extracts, obtained by dialysis and by filtration, as compared with that of soil suspensions

SOIL NUMBER	SOIL	H-ION CONCENTRATION OF SUSPENSIONS (ELECTROMETRIC)	H-ION CONCENTRATION OF DIALYSATE (COLORIMETRIC)	H-ION CONCENTRATION OF FILTERED EXTRACT (COLORIMETRIC)
		pH	*pH*	*pH*
1	Norfolk sandy loam	4.85	4.95	4.85
12	Decatur silt loam	5.17	5.15	5.10
16	Gray silt loam	7.10	7.10	7.00
17	Norfolk sandy loam	4.92	5.00	5.00
18	Norfolk sandy loam	5.87	6.00	6.00
19	Norfolk fine sand	6.42	6.35	6.40
20	Decatur silt loam	5.61	5.60	5.60
21	Wooster silt loam	6.66	6.85	6.80
22	Ruston sandy loam	5.11	5.15	5.00
23	Norfolk sandy loam	5.99	6.05	6.00
24	Houston clay	7.88	7.80	7.80
25	Decatur silt loam	4.46	4.55	4.50

been used, but the use of flocculating agents with subsequent filtering seems to have become the most general procedure. Harper (10) has recently made a study of the method and gives a good review of the literature. After making a comparison of various flocculents used, he recommends the use of a combination of copper sulfate, calcium oxide, and magnesium carbonate. The collodion sack method gives clear extracts and seems well adapted for use in nitrate determinations. It seemed desirable, therefore, to compare this method with other methods of obtaining extracts for nitrate determinations.

In table 8 is given the nitrate content of soil extracts as determined by the phenoldisulfonic acid method when the extracts had been obtained by different methods. The figures given are averages of duplicate determinations. The flocculation method used was that recommended by Harper, and the dialysates

were obtained as previously recommended. With the suction filtering method the suspensions were prepared according to the procedure of Stewart (25). Filtration was done with the modified suction filtering apparatus previously mentioned.

As the data indicate, the agreement among the three methods is in most cases good. The flocculation method, however, gives erratic results in a few cases. Upon making duplicate determinations of nitrates on these extracts the flocculation method often gave poorer checks than did the extracts from the other two methods. This was believed to be due partly to the large

TABLE 8

Nitrate nitrogen in soils when the extracts are obtained by different methods

SOIL NUMBER	SOIL	METHOD OF OBTAINING SOIL EXTRACTS		
		Dialysis	Modified suction filtering	Flocculation by Harper method
		p.p.m.	*p.p.m.*	*p.p.m.*
1	Norfolk sandy loam	66.2	67.4	73.4
2	Sumter clay	5.4	6.5	6.6
3	Gray silt loam	8.6	9.9	9.5
4	Greenville sandy loam	48.7	49.3	42.5
5	Dekalb fine sandy loam	25.9	27.2	24.1
6	Brown silt loam	65.7	66.9	73.5
11	Dekalb fine sandy loam	6.3	6.3	7.2
12	Decatur silt loam	10.8	9.9	10.6
13	Greenville sandy loam	42.6	43.2	41.2
14	Greenville sandy loam	4.6	4.7	5.4
15	Black silt loam	25.8	25.7	28.6
	Average	28.2	29.7	29.3

amount of salts present as a result of the reagents added, and partly to the fact that there was often a slight interfering color in these extracts.

The use of flocculents was also found to necessitate the making of a nitrate determination on the reagents. Thus, it was found that c. p. magnesium carbonate contained appreciable amounts of nitrates. On the other hand, certain kinds of filter paper may absorb nitrates, as was shown by Gimingham and Carter (9). The collodion sack method eliminates both of these sources of error, and insures the securing of clear extracts.

The use of decolorizing agents

Beside the slight turbidity of extracts, which is commonly present and which interferes in the phenoldisulfonic acid method of determining nitrates, there is also, with some extracts, the interference of color due to the presence of soluble organic matter. This is usually overcome by the addition of absorbents. As is obvious, absorbents can be used equally well with the collodion sack method as with any other method.

A review of the literature indicates that carbon black and alumina cream are the absorbents most commonly recommended. Chamot and coworkers (4), and later Emerson (7) recommended the use of alumina cream, whereas Schreiner and Failyer (23), Harper (10) and others found "G Elf" carbon black to be satisfactory. In a brief comparison the writers found that carbon black was much superior to alumina cream in decolorizing power. It was also found that alumina cream absorbed appreciable amounts of nitrates. Harper, however, raised the following objections to the general use of carbon black; (*a*) the passing of finely divided carbon through the filter paper which caused a black residue upon evaporation, (*b*) the possibility of the carbon black absorbing more or less nitrate, (*c*) the inability of carbon black to flocculate soil suspensions, and (*d*) the difficulty of removing it from the apparatus. It will be noticed that the first and third objections are removed when the collodion sack method is used. It was also found that the sacks could readily be cleaned practically free of carbon black.

TABLE 9

Nitrate nitrogen in soils when various kinds and various amounts of carbon black are added to the soil suspension in the collodion sack

SOIL NUMBER	SOIL	TREATMENT WITH CARBON BLACK				
		None	0.2 gm. "Pfanstiehl"	0.5 gm. "Pfanstiehl"	0.2 gm. "G Elf"	0.5 gm. "G Elf"
		p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
3	Gray silt loam	8.3	7.2	5.0	8.2	8.4
6	Brown silt loam	64.6	62.6	60.5	62.2	64.8

A brief study was made of the possibility of absorption of nitrates by two different kinds of carbon black. The carbon black was added to the soil inside the sack (20 gm. of soil) and the nitrates were determined in the dialysate after the usual 18 hours standing. The results are presented in table 9. It is evident that the "Pfanstiehl" decolorizing carbon will absorb nitrates from some soil extracts if used in large amounts, while the "G Elf" has no effect.

In working with a compost soil giving a highly colored extract 0.2 gm. of carbon black added to 20 gm. of soil was found sufficient to decolorize the extract. Therefore, in working with soils giving colored extracts it is recommended that 0.2 gm. of "G Elf" carbon black be added to the soil inside the sack.

PHOSPHORUS AND CALCIUM DETERMINATIONS

The collodion sack method of obtaining soil extracts was used with advantage by Parker and Tidmore (20) in the determination of phosphorus in the soil extract. They allowed 18 hours for solution and diffusion to take place before they analyzed the dialysate. Since the solution of soil phosphorus, calcium,

and other mineral soil elements is very gradual and continuous, as was pointed out in the equilibrium studies in table 4, an arbitrary period of time for solution and diffusion must be selected. Table 4 indicates that 18 hours is a very satisfactory period to use. It then seemed desirable to compare this procedure with other methods of securing extracts.

Table 10 gives the concentration of phosphorus and calcium and the specific resistance of soil extracts obtained by the collodion sack method, the modified suction filter method, and the Pasteur-Chamberland filter methods. In the latter two methods the soil suspension was given the preliminary mixing used

TABLE 10

*The specific resistance and the phosphorus and calcium concentration of the soil extracts obtained by different methods**

SOIL NUMBER	SOIL	METHOD	PHOSPHORUS	CALCIUM	SPECIFIC RESISTANCE
			*p.p.m.**	*p.p.m.*	*ohms*
7	Gray silt loam	Collodion sack	12.10	67.8	8,120
		Suction filter	13.40	76.5	7,060
		P. C. filter	9.90	67.0	7,960
8	Gray silt loam	Collodion sack	3.10	40.0	10,100
		Suction filter	3.40	40.8	9,160
		P. C. filter	2.70	33.9	9,740
9	Norfolk sandy loam	Collodion sack	1.00	71.3	5,220
		Suction filter	1.00	74.8	5,220
		P. C. filter	0.50	74.8	5,220
6	Brown silt loam	Collodion sack	0.75	68.7	6,640
		Suction filter	Trace	76.5	8,120
		P. C. filter	Trace	62.6	8,450
10	Norfolk sand	Collodion sack	2.10	40.0	13,960
		Suction filter	2.20	40.8	14,620
		P. C. filter	1.40	33.0	16,390

* Calculations made on basis of dry soil.

by Stewart (25) in his soil extract studies. One-half the suspension was filtered through a Pasteur-Chamberland filter and the other half through the modified suction filter. The dialysate was obtained after 18 hours, the hand method of shaking being used. The phosphorus was determined by the Coeruloemolybdate colorimetric method used by Parker and Tidmore, and the calcium was determined by the ordinary volumetric method. Since in most cases only 100 cc. of extract was used for calcium determinations, the minor discrepancies noticed in the table can be accounted for.

The data in table 10 show that the collodion sack method and the suction filter method give extracts of practically the same concentrations of phosphorus

and calcium. It is evident, therefore, that the 18-hour dialysis gives as good an arbitrary method of obtaining soil extracts for the study of the water-soluble mineral constituents of the soil as do the ordinary filtering methods. As is again shown, the Pasteur-Chamberland filters may absorb appreciable amounts of phosphorus.

It is evident from these studies that the collodion sack method is not only adaptable to the securing of extracts for determinations of H-ion concentration, of nitrates, and of water-soluble phosphorus and calcium, but also that it should be equally well adapted for use when any of the other water-soluble constituents of the soil are to be determined. Not only does it furnish large amounts of clear extract from which all water-soluble constituents can be determined by even the most exacting colorimetric methods, but also its use is simple. Moreover, as used by Parker and Tidmore (20) it furnishes a convenient method of studying the solubility of soil constituents.

SUMMARY

The advantages and disadvantages of different methods of obtaining soil extracts are considered. The use of collodion sacks in obtaining soil extracts by dialysis seems to offer a method that is short and simple and gives the soil extract in an unaltered condition. The method was studied in detail.

1. By a procedure given in detail, twenty collodion sacks can be made easily in one hour.

2. A sack may be used several times in preparing soil extracts. Neither old nor new sacks influence the composition of the extract.

3. Equilibrium studies on the dialysate and on the filtered soil suspension from the inside of the sacks, obtained after various periods of contact and with various methods of shaking of the system, may be summarized as follows:

a. Continuous mechanical shaking of the dialyzing system or flasks for 1 hour gave a quicker diffusion of ions through the membrane than did hand shaking at intervals of 15 minutes for the same period.

b. For ions that come into solution quickly, 2 hours of shaking of the flasks in a drawer type shaker, or 18 hours standing with occasional hand shaking was found to establish complete equilibrium. Both of these methods of shaking are recommended as practical.

c. For ions that come into solution slowly, the arbitrary time of 18 hours of standing, with hand shaking of the flasks once every hour during the day, is recommended. This procedure was found to give extracts of practically the same concentration of phosphorus and calcium as does the common method of obtaining extracts.

4. A study of the collodion sack method of obtaining soil extracts for the determination of nitrates, H-ion concentration, and water-soluble phosphorus and calcium established the following points:

a. The H-ion concentrations of soil extracts obtained by the collodion sack method, of filtered soil extracts determined by the colorimetric method, and of soil suspensions determined by the electrometric method agree well. The collodion sack method, therefore, is of special value where the H-ion concentration of soils is to be determined by the colorimetric method.

b. The buffer capacity of soils can be determined readily by means of the "Dialysis-colorimetric" method.

c. Extracts obtained by the collodion sack method have the same concentration of nitrates as do extracts obtained by filtering or flocculation methods.

d. "G Elf" carbon black completely removes the color of extracts due to soluble organic matter without affecting the nitrate nitrogen content of the extract.

e. The collodion sack method not only offers a new simple method for use in the determination of the water-soluble constituents of the soil but also offers a means of studying the solubility of the relatively insoluble soil constituents.

5. The advantages of the collodion sack method over other methods of obtaining soil extracts may be briefly summarized as follows:

a. It gives clearer soil extracts than is usually obtained by other methods, and is, therefore, especially adapted to the determination of the water-soluble constituents of the soil by colorimetric methods.

b. It does not affect the constituents of the soil extract as does filtering with Pasteur-Chamberland filters and in some cases with filter paper.

c. It requires no flocculating agents, and thus eliminates any error due to the addition of flocculents.

d. It requires little apparatus, is simple and inexpensive, and is well adapted to routine laboratory work where large numbers of extracts are to be obtained.

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THE OCCURRENCE OF YEASTS IN SOIL

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INTRODUCTION

Besides the numerous bacteria and actinomyces and a few molds that develop upon the plate from soil, there may occur occasionally a colony of a yeast. Plates for counting bacteria and actinomyces, however, are generally made at relatively high dilutions of soil (100,000 to 10,000,000) and it would not appear surprising if yeasts failed to appear in abundance at these dilutions even though they were numerous in the soil.

The general occurrence of yeasts in considerable abundance on fruits and flowers suggests their presence in soils. That they do occur there has been repeatedly shown, but in what abundance may they be expected? Previous studies on the occurrence of yeasts in nature give little information on the abundance with which they may be found in soil or what species may be most frequently encountered. There appears to have been no systematic study conducted to determine whether they occur sufficiently regularly or abundantly to be factors of importance in soil transformations, whether they should be considered normal soil inhabitants or occasional invaders.

The following studies were conducted to cast light upon the presence of yeasts in soil, to determine how widely they may be found, in how great a diversity of forms, and in what abundance. It was of particular interest to determine whether yeasts might be considered as important elements of the microscopic population of the soil.

HISTORICAL

The extensive researches of Hansen on yeasts embraced studies on the cycle of some yeasts in nature, particularly *Saccharomyces* (*Hansenia*) *apiculatus* and some of the true *Saccharomycetes*. In the early studies on the apiculate yeasts, Hansen (12, 13) determined that these forms depended principally upon the sweet, ripe, juicy fruit for their proliferation. Developing on the fruit they would become disseminated principally by air currents and also by insects and rainfall to other fruits and soil. The fallen fruit also carried yeasts to the soil. They could persist for some time in soil but apparently did not find conditions particularly favorable for development. It was believed that they lived over the cold months in soil and were introduced upon fruit again from soil by dust, insects, and other natural agencies. Accordingly it was noted that these yeasts were more abundant during the fruiting season and less numerous in the spring. Their numbers also increased in the atmosphere during the late

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summer (14). When yeasts (*Saccharomyces apiculatus*, *S. pastorianus*, *S. ellipsoideus*, Carlesberg No. 1, and a brewery yeast) were inoculated into soil and kept under natural conditions they persisted for at least a year although their numbers decreased (15). *S. apiculatus* was still found in the inoculated soil after exposure to natural conditions for three years.

It was later shown (16, 17, 18) that the true *Saccharomycetes* pass through a cycle much the same as the apiculate yeasts, but because the true *Saccharomycetes* form spores and *S. apiculatus* does not, the former persisted longer and appeared more widely distributed. Since the most important region of development of these yeasts is fruit, it is not surprising that they were found more abundantly in soils of vineyards and orchards and less frequently at distances from these regions. *Saccharomycetes* were found in 67 per cent of samples of soil under fruit trees, in 30 per cent of samples of soil in neighboring woods, and in 19 per cent of samples in remote fields. Soils of mountainous regions contained extremely few *Saccharomycetes*. The true *Saccharomycetes* were found more widely distributed than the apiculate yeasts, and *Willia* and *Pichia* forms were more tolerant to natural climatic conditions than the true *Saccharomycetes*. Although these data suggest that these forms may occur temporarily in soil they do not indicate that yeasts are of importance in soil transformations.

Many of these observations were verified or amplified by Berlese (2), Müller-Thurgau (29, 30), Wortmann (37), Cordier (7), Klöcker (22), Boutroux (3, 4, 5), and Heinze (19). [See also Klöcker (23) and Guilliermond (11).]

Müller-Thurgau (29) determined that wine yeasts occurred in orchard and vineyard soils not only at the surface but even at 20 to 30 cm. below the surface. None were found at a depth of 40 cm. Berlese (2) found them in vineyards and orchards down to 12 or 13 cm.

Fischer (9) found that yeasts were often favored by soils rich in humus and found them particularly abundant in high moor soils. Klöcker (22, 21, 25, 24) isolated many new species of yeasts from soils from various parts of the world. These included *Saccharomyces*, *Endomyces*, *Debaromyces*, and *Schwanniomyces*. Ludwig (27), DeKruyff (8), and Adametz (1) obtained yeasts from soil.

Pasteur early showed that yeasts did not occur abundantly on green fruit (32, 35, 31) but developed rapidly on the ripened fruit if no precautions were taken to prevent their deposition on the fruit. Yeasts have been found in considerable abundance in flowers by Boutroux (3, 4, 5), Berlese (2), Cordier (7), Schuster and Vladimir (34), and Reukauf (33). Several of these authors have verified Pasteur's observations on the absence of yeasts on immature fruit.

That insects may be important factors in transporting yeasts from fruit to fruit and from flower to flower is quite generally accepted (17, 29, 37, 22, 3, 5, 4). In these instances the yeasts may be merely carried on the surfaces of the insects' bodies or pass through their digestive systems. Brefeld (6) and Berlese (2) considered that yeasts might not only multiply in the digestive tracts of some insects, but might have their principal abode there.

As regards the occurrence of *Torulae* in soil there appears to be very scant information. Adametz (1) found white as well as red species widely distributed. DeKruyff (8) found that the *Torulae* composed a comparatively small proportion of the total number of yeasts found in soil and foliage. Ludwig (27) isolated some *Torulae* from soils of the Alps. Klöcker (22) cites Hansen as finding species of *Torula* distributed widely in nature. Kohl (26) states that the greatest number of yeasts which are found in nature on fruit or in soil are wild yeasts.

Most of these researches appear to have been primarily concerned with the sources of yeasts that occur on fruit and not with the importance of yeasts as representatives of the soil flora.

METHODS

For obtaining the soil samples, sterile cylindrical samplers were used (10.5 cm. long by 2 cm. inside diameter). Samples were taken to a depth of $6\frac{2}{3}$

inches after the litter on the soil surface was removed. The soil was removed from the samplers by sterile wooden plungers to sterile 250-cc. bottles. These samples, which weighed from 20 to 50 gm., were suspended in 100 cc. of sterile tap water. One-cubic centimeter portions of these suspensions (equivalent to 0.2 or 0.3 gm. of soil) were inoculated into 100 cc. of a medium composed of 90 cc. nutrient broth and 10 cc. dextrose-tartaric acid solution. The dextrose-tartaric acid solution was composed of 50 per cent dextrose and 5 per cent tartaric acid. This solution was sterilized separately from the broth and added after sterilization. The final reaction of the medium was close to pH 3.8. This acidity prevented the development of bacteria but supported growth of yeasts and filamentous fungi. The molds tended to form a heavy pellicle upon the surface. In order to obtain samples relatively free of molds from these cultures, flasks were used which had a tap sealed in at the bottom. If yeasts developed they could be obtained readily in samples drawn off through this tap after incubation for about a week. After the incubation period the samples were diluted and plated upon an agar medium composed of 9 cc. nutrient agar and 1 cc. of the above mentioned dextrose-tartaric acid solution. Subsequent to incubation for 24 or 48 hours, the plates were examined for yeast colonies which were planted upon Sabouraud's agar of the following composition:

	per cent
Peptone	1
Maltose	4
Agar	1.8

The cultures so obtained were studied morphologically and culturally for identification.

As alternative media for initial enrichment and plating, the medium suggested by Waksman (36) for plating filamentous fungi proved as satisfactory as the above. The solution medium was of the following composition:

	per cent
Dextrose	1
Peptone	0.5
KH_2PO_4	0.1
$MgSO_4$	0.05

About 10 cc. sterile 1/15 *N* H_2SO_4 was added to 90 cc. of this sterilized medium to bring the pH to 3.8. A solid medium was prepared containing 3 per cent agar. Just before pouring the plates 1 cc. of sterile 1/15 *N* H_2SO_4 was added to each 10 cc.

In some instances soil samples were plated directly upon the acidulated agar without previous enrichment in the solutions, and yeast colonies were picked after incubation. In some instances 100-gm. portions of soil were incubated after the addition of 1 or 2 gm. of dextrose. Later these were either plated or enriched in the nutrient-dextrose-tartaric acid solutions.

RESULTS

In all, 87 different soils, obtained principally from a region within a radius of 50 miles of Minneapolis, Minnesota, were studied. Of these, 59 were obtained from Minnesota, 9 from Wisconsin, 16 from New Jersey,[2] and 3 from New Hampshire. Included among these were sands, peats, clays, and muck supporting such a variety of vegetation as brush and trees, grasses, grain, garden truck, and fruit.

It was strikingly apparent throughout the studies that the soils harbored extremely few yeasts. Of the 87 soils studied, 48 (55 per cent) yielded no yeasts by any of the methods applied, whereas 39 (45 per cent) gave one or more species. Only 2 soil samples, 1 lawn and 1 garden, yielded three different species of yeast each. From 10 samples of soil two different species of yeast were obtained.

From no single sample of soil were more than three different species of yeast obtained, although several different methods were used to obtain them.

Molds occurred in abundance from most soils whether the soils were enriched in the acidulated sugar medium before plating or whether the soil was plated directly. It was particularly striking that the molds occurred in great abundance, whereas the yeasts were scant. In two cases where the yeasts appeared fully as abundantly as in any soil there occurred 26,000 and 7,600 molds per gram, whereas only a few colonies of yeasts were found. In this instance suspensions equivalent to 1/4 or 1/40 gm. of soil were plated for the yeasts. Dilutions of over 1/100 gm. of the original soil would scarcely ever show yeast colonies when plated or inoculated into liquid media. Even when a suspension equivalent to 1/10 gm. of soil was used in direct plating there seldom developed over five colonies of yeasts. Plate counts of yeasts should, moreover, give fairly good representations of the abundance of yeasts due to the type of their reproduction. These results would appear to indicate definitely the relative scarcity of yeasts in soil.

Because the yeasts appeared in such small numbers in any of the soils used, even including those supporting large and small fruit stock, there chould hardly be very much importance attached to correlations between the finding of yeasts and the vegetation, reaction, soil type, or seasonal variation except as such data might show a haphazard distribution under the various conditions or a consistent presence or absence under some conditions. The following tables indicate the instances where yeasts were obtained and where they were not found.

[2] The authors are very grateful to Dr. Waksman of the New Jersey Agriculture Experiment Station at New Brunswick and to Mr. Jones of the experimental cranberry laboratories at Whitesbog, New Jersey, for providing soils growing cranberries and blueberries. Thanks are also extended to Prof. Blair for permission to obtain samples from the experimental plots at the New Jersey station and to Professor Rost for permission to obtain samples from the experimental plots at the University of Minnesota.

Table 1 shows a correlation between the occurrence of yeasts in soil and the plant growth. Of the 45 cultivated soils, 17 (38 per cent) gave cultures of yeasts. There appears to be no significant difference whether these soils grew legumes or non-legumes, grasses or garden truck. The proportion of soils

TABLE 1

Correlation between the occurrence of yeasts in soil and the vegetation on these soils

NUMBER OF SOILS	VEGETATION	NUMBER OF YEASTS FOUND	NUMBER OF SOILS WITH ONLY ONE YEAST	NUMBER OF SOILS WITH MORE THAN ONE YEAST	PER CENT OF SOILS WITH YEAST
45	*Cultivated*	22	13	4	38
33	Grasses and grain	13	11	1	36
6	Legumes	2	2	0	33
5	Mangels and potatoes	5	0	2	40
1	Gardens	2	0	1	100
3	*Lawns*	7	0	3	100
5	*Pasture and prairie*	2	2	0	40
19	*Orchards and small fruits*	13	7	3	53
9	Large fruits	5	1	2	33
4	Berries and grapes	2	2	0	50
2	Blueberries	2	0	1	50
4	Cranberries	4	4	0	100
8	*Wooded Land*	3	1	1	25
3	*Sand Dunes and Banks*	3	3	0	100
4	*Swamps and Marshes*	3	1	1	50
87		53	27(31%)	12(14%)	45

TABLE 2

Correlation between soil type and the occurrence of yeast

SOIL TYPE	SOILS WITH YEASTS		SOILS WITHOUT YEAST		TOTAL NUMBER OF SOILS
		per cent		*per cent*	
Muck	0	0	2	100	2
Peat	4	80	1	20	5
Sand	6	67	3	33	9
Sandy loam	13	38	21	62	34
Clay loam	11	35	20	65	31
Clay	5	83	1	17	6
Totals	39	45	48	55	87

showing yeasts was nearly the same in all instances. Included in this group of soils were 9 samples from experimental plots of New Jersey, 2 of which gave yeasts, and 9 samples from experimental plots of Minnesota, none of which gave yeast development.

The three lawn soils each gave more than one yeast, a result which appears suggestive in spite of the fact that the data are too scant to carry much weight.

Only 2 of 5 soils of pastures and prairie types gave yeasts, whereas practically half of the orchard soils gave yeasts. This low proportion was rather unexpected in the light of the results of other workers, particularly since many of these soils were studied during September, a period when yeasts should occur in these regions in the greatest abundance. The percentage of soils growing

TABLE 3

Correlation between reaction of soils and occurrence of yeasts

SOIL REACTION	SOILS WITH YEASTS		SOILS WITHOUT YEASTS		TOTAL NUMBER OF SOILS
pH		*per cent*		*per cent*	
4.0–4.5	4	100	0	0	4
4.6–5.0	1	50	1	50	2
5.1–5.5	2	40	3	60	5
5.6–6.0	0	0	7	100	7
6.1–6.5	9	50	9	50	18
6.6–7.0	10	45	12	55	22
7.1–7.5	1	20	4	80	5
7.6–8.1	4	44	5	56	9
Totals.....	31	43	41	57	72

TABLE 4

Correlation between season of year and occurrence of yeasts in soil

MONTH	SOILS WITH YEASTS		SOILS WITHOUT YEASTS		TOTAL NUMBER OF SOILS
		per cent		*per cent*	
May.....	6	24	19	76	25
July.....	1	33	2	67	3
August.....	6	40	9	60	15
September.....	9	43	12	57	21
December.....	5	22	18	78	23
Totals.....	27	31	60	69	87

large fruits which showed yeasts (33 per cent) was much below that of the soils growing cranberries (100 per cent). The reaction of the last mentioned soils was considerably below that of the soils growing large fruits. Only 2 of 8 soils of woodland showed yeasts. Each of the three samples of sands developed yeasts. Only 2 of 4 samples from swamps and marshes showed yeast development.

Table 2 presents a correlation between the occurrence of yeasts and the type of soil roughly classified under six types. Yeasts were found in practically

all soils except muck. In this instance but two samples were studied, consequently these results would hardly be significant. In peat and clay a particularly high proportion of the samples yielded yeasts (80 and 83 per cent respectively). Sands also gave a high proportion. The results as a whole, however, show little that could be taken as indicative that yeasts would appear more abundantly in soils of one type than another.

Even though yeasts were found between pH 4.0 and 4.5 in all of the four soils and between pH 6.0 and 7.0 in only 60 per cent of the cases, there was no regularity in the results to indicate that the abundance of yeasts might be affected by alkaline or acid conditions. (See table 3.)

Table 4 indicates that yeasts might be found more commonly during the late summer than during the early summer or the winter.

The results as a whole indicate that the yeast population is in general so small that the effects of plant growth, soil structure, soil reaction, or seasonal variations have slight significance. The differences which appear in these results are more like chance scattering than distribution significantly correlated with environmental factors. The conclusion seems justifiable that yeasts occur in most soils in such small numbers as to be of little importance in the transformations that occur there.

DESCRIPTION OF SPECIES

The classification and identification of yeasts are not easy. This is more particularly true of the species, for most strains may be readily placed in their proper genus after a little study; but the published descriptions of species are frequently so incomplete, or their identification is based upon such variable characters, that it is almost impossible to decide whether an organism is new or one that has been previously described. It has been possible to identify positively but three of the twelve species isolated—*Nadsonia fulvescens*, *Willia anomala*, and *Torula glutinis*.

Several of the organisms isolated proved on further study not to be true yeasts, the yeast-like cells which were found on agar plates later developing mycelium. The authors were thus misled three times by the sporidia of *Ustilago zeae;* and once each by an organism closely resembling *Oidium lactis* and another very similar to *Monilia candida*, the latter producing mycelium only under semianaerobic conditions in agar stab cultures. *Chlamydomucors*, producing budding yeast-like cells and an alcoholic fermentation, also proved misleading at first.

The forty-nine strains of yeasts isolated have been observed in cultures grown on Sabouraud agar, and in large tubes of 5 per cent dextrose broth. Further study of fermentation was made in small tubes containing 1 per cent sugar broths with gas traps and Andrade's indicator; dextrose, lactose, sucrose, maltose and mannite were used. Each strain was tried three times for gelatine liquefaction, once in ordinary gelatine stabs, once in 5 per cent dextrose gelatine stabs, and once by the method recently described by Frazier (10); in no

case, however, was any digestion of the gelatine shown. All cultures were incubated at about 30°.

Morphological characters were determined from young (24-hour) Sabouraud agar cultures, and from the same cultures after they were a week or more old. For spore formation, the classical plasterblock method was used, and, with much more success, the carrot infusion calcium sulfate agar recently described by McKelvey (28). Spores were also found in old agar cultures in several instances. Morphological characters have been determined almost entirely by the examination of unstained wet preparations. The identification of spores has been confirmed in every instance by the use of Moeller's spore stain, and fat globules have been identified by the use of Sudan III and osmic acid.

It has been impossible positively to identify most of the species. However, it has appeared particularly striking that the various species maintained with marked constancy even slight differences in morphological and cultural characters over relatively long periods of subcultivation; in particular, differences in morphological characters consistently correlated with cultural characters. The twelve species were first separated almost entirely on morphological characters, but subsequent tabulation of cultural characters showed that those which were identified as the same by morphological characters had, with few exceptions, the same cultural characters.

In grouping the species isolated, the classification and nomenclature of Guilliermond (11) has been followed. The twelve species are described below, and are illustrated in the accompanying plate. The various figures are all camera lucida drawings at the same magnification. In figures 1 to 8, inclusive, the upper half shows the morphology in old cultures or cultures from McKelvey's medium for spores; the lower half shows the cells in young Sabouraud agar cultures. In the remaining figures the entire field shows cells from a young culture, the old cultures not showing any noteworthy difference.

Zygosaccharomyces sp. (fig. 1). This yeast, found but once, was the only species isolated showing definite evidence of conjugation preceding spore formation. The vegetative cells are oval and of medium size. Spores were formed readily on plaster blocks, on McKelvey's medium, and in old Sabouraud agar cultures. Practically all of the asci were of dumbbell shape, showing that conjugation had taken place; but one of the gametes was almost invariably smaller than the other. The conjugation is therefore heterogamic. From two to four spores are formed, three being the most frequent number. The spores are round. This species differs from the described species in failing to produce fermentation of any of the sugars tried. In dextrose broth a sediment is formed, after some days a very thin incomplete pellicle develops.

Nadsonia fulvescens (fig. 2). This is quite the largest yeast encountered. The vegetative cells are of various shapes, but pear-shaped cells with a small nipple-like projection at one end, similar to those which occur at both ends in *Hansenia apiculata*, are very numerous and characteristic. The protoplasm contains numerous fine highly refractile granules, very rarely a vacuole. The growth in young agar cultures is white and very mucoid in character. As the culture grows older the growth becomes tan and then gradually reddish brown; one strain became almost orange in color. This color change is evidence of spore formation. Guilliermond (11) describes the spores as containing a large fat globule and having a roughened

membrane. The fat globules were found quite regularly, and in some cultures the outer lamella of the thick spore wall peeled off in places giving a very irregular contour. Spores are supposed to be the result of a heterogamic conjugation of a cell (macrogamete) with its bud (microgamete). A considerable number of the sporogenous cells showed an attached bud. Guilliermond (11), reports that this species slowly ferments several sugars, which was not found to be the case with any of these strains. But in morphological characters, in the color of the colonies, and in the characteristic development of white (non-sporogenous) sectors in old colonies it seems identical with *Nadsonia fulvescens*. This yeast was isolated from six different soils.

Torulaspora sp. (fig. 3). The young cells are small and round, without granules and only occasionally showing a vacuole. The sporogenous cells are larger, contain a very prominent large fat vacuole, and in the majority of cases send out spurs which are looked upon as copulation tubes; in no case, however, was evidence of conjugation found. Only one strain was isolated, which gave a dull white growth on Sabouraud agar, and a heavy pellicle on dextrose broth, without fermenting any of the sugars.

Saccharomyces sp. Two species of spore-forming yeasts producing an alcoholic fermentation were isolated. These are designated *A* and *B*.

Saccharomyces A. (fig. 4). The young cells are of medium size and oval. Nearly all contain a large vacuole with a "dancing body," and numerous small granules and vacuoles. Spores were formed rather scantily on plaster blocks, fairly abundantly—two to four—on McKelvey's medium. Agar slant cultures gave a rather restricted growth, moist and shiny, white at first but rapidly becoming brown. Only dextrose was fermented; this yeast therefore belongs in the fourth sub-group of the genus. No pellicle was formed on liquid media. This species was isolated twice.

Saccharomyces B. (fig. 5). The young cells are much larger and rounder than those of the preceding species, resembling *S. cerevisiae*. They also possess very large volutin vacuoles, and numerous small granules. Two to four spores are formed readily on plaster blocks, on McKelvey's medium, and in old agar slant cultures in occasional cells. The growth on Sabouraud agar slant cultures is abundant, moist and white, remaining white for some weeks. In 5 per cent dextrose broth a very vigorous alcoholic fermentation with abundant gas is produced; no pellicle is formed. One strain of this species was found to produce 12 per cent alcohol. Sucrose is also fermented, but none of the other sugars tested is. It therefore belongs in the second sub-group of the genus. It was also isolated twice.

Pichia sp. (fig. 6). The young cells are large, oval to sausage-shaped, and contain large vacuoles. Spores are formed readily in old cultures, on plaster blocks and on McKelvey's medium. They vary from two to four in number, three being found most frequently. The spores are round and each contains a highly refractile fat globule characteristic of the spores of this genus. On Sabouraud agar an abundant spreading dry growth with a dull surface is formed. On dextrose broth a heavy dry scum imprisoning gas bubbles appears. Gas is produced abundantly but rather slowly with most strains. Sucrose is also fermented. This species, isolated from six different soils, could be readily recognized by the strong odor of banana oil (amyl acetate) produced in all cultures with fermentable sugars. This is so striking that the authors believe this species would have been recorded if it had been described previously. Sartory [quoted by Guilliermond (11)] has described a yeast much like *Willia saturna*, isolated from banana leaves, which gave a banana odor.

Willia anomala (fig. 7). This species was readily identified by the hat-shaped spores which were formed on McKelvey's agar and on plaster blocks. The growth on agar was thinner than that of the preceding species; one strain showed a tendency to produce mycelium dipping down into the agar in old cultures, indicating the close relationship to *Endomyces*. On dextrose broth, a thin pellicle was formed and fermentation was rapid and abundant; sucrose and maltose were also fermented. All strains gave the characteristic ethyl acetate odor. This species was found three times.

Torula sp. The majority of the yeasts isolated fell in this genus, i.e., they failed to produce spores, or scums on liquid media, and for the most part gave no fermentation. The descriptions of the many species which have been referred to this genus are particularly incomplete and confusing. One species, *Torula glutinis*, was readily recognized by its characteristic pigment. The remaining varieties have been grouped together as species, but little attempt to identify them has been made because of the hopeless confusion in the literature. In addition to those named, four different species were recognized which have been designated *A*, *B*, *C*, and *D*.

Torula glutinis (fig. 8). Four strains of yeasts producing a red pigment were isolated from as many different soils. In morphological and cultural characters, these all proved identical with each other and with a strain of *Torula glutinis*, obtained from the American Type Culture Collection, also with a strain isolated from oysters which has been completely described by Hunter (20). In spite of considerable confusion in the literature on red yeasts, it is believed that these strains can be positively identified. The young cells are small and oval, and are relatively free from vacuoles and granules. In old cultures the cells contain a large fat globule. Agar slant cultures are abundant and very mucoid in character. After a time most of the growth slides to the bottom of the tube. In liquid media some turbidity and an incomplete ring pellicle are produced. No fermentation occurs with any of the sugars.

Torula A. (fig. 9). This yeast was found but once. The cells vary in form, an elongated oval being the predominating type. Reproduction appears to be by a process intermediary between budding and fission, as in *Saccharomycodes*. The cells contain numerous small, highly refractile fat globules which increase in number with the age of the culture but show no tendency to coalesce. Agar slant cultures are somewhat restricted, smooth and white. In dextrose broth turbidity and sediment are produced without fermentation. None of the other sugars were fermented.

Torula B. (fig. 10). This was obtained from five different soils. The young cells are large and oval, with prominent volutin vacuoles and especially striking "dancing bodies" many of which are of the size and shape of small bacilli. In older cultures the cells are smaller, and more variable both in size and form. Agar slant cultures are smooth and white. In 5 per cent dextrose broth a fairly rapid and abundant fermentation is produced. None of the other sugars is fermented.

Torula C. (fig. 11). The cells are smaller than the preceding, and nearly round. Agar slant cultures are smooth and white. No fermentation occurs with any of the sugars. This yeast was isolated twice.

Torula D. (fig. 12). In this group have been placed sixteen strains isolated from as many different soils, which showed some slight differences, but all of which were characterised by very small cells and practically no fermentation. The illustration is a composite field from young cultures of several different cultures, and shows the degree of variation in morphological characters. In some strains round cells were more numerous, in others elliptical ones, but in no case were these characters sufficiently prominent or constant to warrant making a new group. In old cultures in general the cells were more variable in size and form, and many contained large fat globules. Agar slant cultures are smooth and white. Twelve of the strains gave a very slow and slight fermentation of dextrose, the gas traps being half filled after about a week. Another produced acid from sucrose. Otherwise there was no fermentation.

SUMMARY

Yeasts were found in small numbers in 39 of 87 soil samples. There was no correlation between the occurrence of yeasts and the type of soil, the nature of the crop, or the season of the year. They were found in such small numbers and so haphazard in distribution as to indicate that they play no important part in soil transformations.

Forty-nine strains isolated fell naturally into twelve groups or species. Of these but three, *Nadsonia fulvescens*, *Willia anomala*, and *Torula glutinis*, could be positively identified. The remaining strains were species of *Zygosaccharomyces*, *Torulaspora*, *Saccharomyces* (two species), *Pichia*, and *Torula* (four species).

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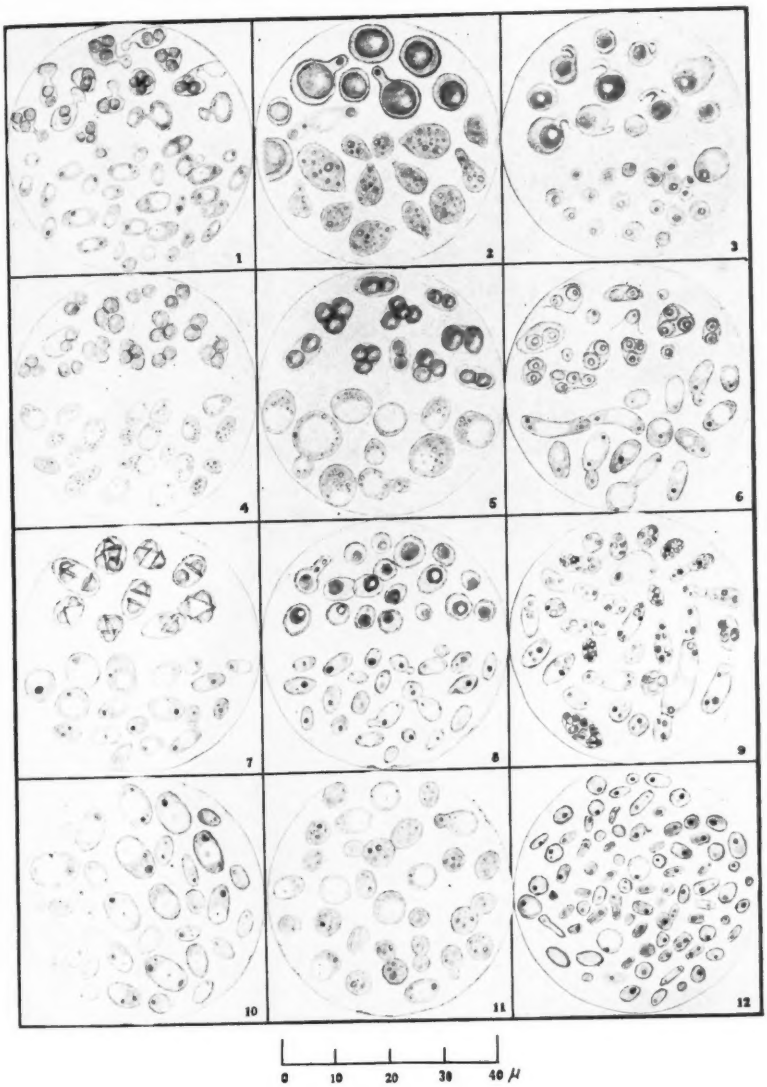
PLATE 1. TWELVE SPECIES OF YEASTS FOUND IN SOILS

In figures 1 to 8, inclusive, the upper half shows the morphology in old cultures or cultures from McKelvey's medium for spores; the lower half shows the cells in young Sabouraud agar cultures. In the remaining figures the entire field shows cells from a young culture.

1. *Zygosaccharomyces* sp.
2. *Nadsonia fulvescens*
3. *Torulaspora* sp.
4. *Saccharomyces* A.
5. *Saccharomyces* B.
6. *Pichia* sp.
7. *Willia anomala*
8. *Torula glutinis*
9. *Torula* A.
10. *Torula* B.
11. *Torula* C.
12. *Torula* D.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STUDIES ON SOME OBLIGATE THERMOPHILIC BACTERIA FROM SOIL[1]

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The investigations of thermophilic bacteria by Barlow (1), Weinzirl (18), Donk (5), and Bigelow and Estey (3), have developed a new interest in this group of organisms because of their significance in the spoilage of canned foods. Thermophilic bacteria have a wide distribution in nature, and their isolation from water, sewage, snow, canned foods, milk, cotton, hay, manure, and soil has been reported by various investigators. As Morrison and Tanner (14) (15) have recently published a careful review of the literature dealing with thermophilic organisms, no historical resumé of this group of bacteria will be presented.

Since two important groups of food products are known to be subject to contamination by thermophilic bacteria, the natural habitat of this group takes on very considerable significance. The literature contains many references to isolations of thermophiles from various sources, but the descriptions of these organisms are so varied and incomplete as to render accurate classification and identification impossible. It is necessary, then, to select the probable habitat of this group and study as many different species as possible, at the same time obtaining a complete and accurate cultural description of each. Only in this manner can the significance and importance of the group be brought into its true position. Nearly all canned foods, as well as milk, are farm products and are intimately related to the soil. It is logical, therefore, to believe that the soil, which is known to contain large numbers of these organisms, is one probable source of the group. The following investigation was undertaken to determine the various types of thermophilic bacteria found in the soil and to obtain an exact cultural description of each of the species isolated.

Twelve samples of the topsoil were collected from fields supporting various types of vegetative growth. Topsoil was used because Globig found that the superficial layer of soil contained the maximum number of bacteria. The topmost two inches was scraped off and a small portion of exposed soil was collected in a test tube containing plain broth. No samples were collected from a depth of more than six inches.

[1] From the Department of Bacteriology of the School of Hygiene and Public Health.

The media for aerobic cultivation were prepared according to standard methods, whereas media usually employed for anaerobic cultivation were made by the methods suggested by Kahn (10). The media were inoculated from the growth on a 24-hour agar slant culture and the reactions were recorded as recommended by the committee on the Descriptive Chart of the Society of American Bacteriologists (13).

All the thermophilic organisms studied grew well at 55°C. and failed to grow at 37°C. when incubated for 28 days. They may therefore be regarded as obligate thermophilic bacteria. The cultures were all bacilli and were easily stained with gentian violet, carbol fuchsin, and methylene blue. Hanging-drop preparations, for the observation of motility, were made from peptone water cultures, incubated at 55°C. for 12 hours. Motility of the organisms was more easily observed with the aid of the dark-field microscope. Without exception, the cultures isolated were motile. For staining flagella, Löffler's and Plimmer and Paine's methods (16) were employed repeatedly but without any definite results.

The usual anaerobic media were employed in this investigation to observe proteolytic action and certain peculiar cultural characteristics. Cooked meat, brain, alkaline egg, and egg cube media were inoculated and incubated for 28 days at 55°C.

McLeod and Gordon (12) have classified bacteria according to their sensitiveness to hydrogen peroxide and their production of catalase. The production of catalase by the cultures described in this paper was determined by scraping a platinum loop over the surface of a 24-hour culture and immersing it in a dilute solution of hydrogen peroxide. A rapid evolution of gas indicates the presence of catalase. McLeod and Gordon found that the great majority of aerobic bacteria produce catalase and it is of interest to note that all the aerobic thermophiles described in this study give this reaction.

The cultures were not pathogenic for guinea pigs when injected subcutaneously, intramuscularly, or intraperitoneally, or when fed by mouth.

THERMAL DEATH POINT DETERMINATION

For the determination of thermal death points in this investigation the method set forth by Bigelow and Estey (3) was followed with a few modifications. Nutrient broth tubes were inoculated with pure cultures of the organism to be tested and incubated at 55°C. for 48 hours to insure good growth. The actively growing broth cultures were transferred to flasks containing 100 cc. of nutrient broth and incubated for 7 days at 55°C. and then for 2 days at 65°C. At the end of this incubation period the flasks were heated to 90°C. for 30 minutes to kill any vegetative forms present, cooled immediately, and stored in the refrigerator to prevent the germination of spores. These broth cultures were centrifugalized and washed three times in sterile salt solution and again transferred to sterile flasks. The concentration of the washed spores was determined by plating out dilutions, and the stock

solutions were diluted, when necessary, to bring the concentration to 2,500,000 spores per cubic centimeter of suspension.

The tubes used for the thermal death point determination were of hard glass, averaging 153 mm. in length, 11 mm. inside diameter, and the wall 0.9 mm. in thickness. They were soaked overnight in 0.1 *N* hydrochloric acid, rinsed thoroughly with distilled water, and sterilized in the autoclave with 4 cc. of normal salt solution in each tube. These tubes were inoculated with 1 cc. of the stock suspension of spores, sealed off to an average of 135 mm., and held at a low temperature until ready to be heated.

A De Khotinsky electric bath containing "Crisco" was used to maintain a constant temperature. Before immersing the tubes in the oil bath, the temperature was raised two degrees above the temperature used for the test, to compensate for the loss due to the introduction of the tubes, and two minutes were allowed for the heat to reach the center of the tubes and for the temperature of the bath to become constant. When a temperature above 130°C. was used, it was found necessary to immerse the tubes in a boiling water bath for one minute and allow for a 3-degree fall in the temperature when the tubes were introduced into the oil bath.

After the initial change in temperature caused by the tubes was allowed for, the oil bath was found to remain constant over the period of heating. No greater rise or fall than 0.5° was observed.

The thermal death point of the spores was determined as follows:

The tubes containing the suspension of spores were held at the desired temperature for 15 minutes and were then removed and placed in the refrigerator to cool rapidly. When cool, the salt suspensions were transferred to sterile broth tubes and incubated for 48 hours. The initial temperature used was 100°C. and tests were made at every increasing 5° up to 160°C. Sterility tests were made by inoculating agar slants with the broth cultures and examining for growth after 24 hours' incubation. Tubes which were sterile were further incubated for 7 days, but it was found that 48 hours' incubation was sufficient time for the vegetation of any viable spores that might have survived the heating. Throughout the thermal death point determination the media used were all carefully adjusted to neutral with brom cresol purple.

SPECIES ISOLATED

Thermobacillus diastasius, nov. sp.

Two cultures of the *Thermobacillus diastasius*, nov. sp. type were isolated. They are large, spore-forming rods with rounded ends and have a granular protoplasm when stained. The average dimensions of the rods are 6.0 by 1.0μ. The vegetative cells retain the gentian violet in the Gram stain. Oval spores are formed at the end of the bacillus and measure from 1.0 to 1.25μ in diameter. The cultures are actively motile.

Acid fermentation but no gas was observed in dextrose, maltose, saccharose, salicin, and glycerin broth. These cultures failed to ferment lactose, starch, inulin, mannit, or dulcite. The tests for hydrogen sulfide and indol were negative, and nitrates were not reduced. No change was produced in meat, egg cube, brain, or alkaline egg. Löffler's blood serum was liquefied after 3 days' incubation and gelatin after 4 days'. Litmus milk was coagulated after 48 hours and the litmus decolorized with the exception of an acid zone, about 1 cm.

thick, in the upper portion of the medium. The growth on potato was abundant and spreading.

Thermobacillus diastasius was not pathogenic for guinea pigs.

The thermal death point of the spores was between 130° and 135°C. when exposed for 15 minutes.

Remarks. This bacillus is a true aerobic thermophile isolated from the soil. With the exception of the reduction of nitrates to nitrites, this organism corresponds to *Bacillus thermodiastaticus*, reported by Bergey. The description of Bergey's organism is, however, not complete enough to identify this culture, since he does not include carbohydrate reactions, which are distinctive cultural features of this species.

Thermobacillus vulgaris, nov. sp.

Two cultures of the *Thermobacillus vulgaris*, nov. sp. type were isolated. The organism is rod-shaped with rounded ends and granular protoplasm, and is actively motile. The bacillus averages 3.0 by 0.48μ. The vegetative cells are Gram-negative and form long chains composed of from 2 to 10 members. Spores are readily formed at the end of the rod.

On plain agar slants the growth of this organism was moderate or slight, spreading, flat, and smooth. In nutrient broth there was a slight haze or cloudiness, but no pellicle formation or sediment.

This culture did not ferment dextrose, maltose, lactose, saccharose, salicin, starch, inulin, glycerin, mannit, or dulcite. Nitrates were not reduced, nor was hydrogen sulfide or indol formed. No change in meat, brain, egg cube, alkaline egg, blood serum, or milk was noted. Gelatin was liquefied only after 28 days' incubation.

Cultures of this organism were not pathogenic for guinea pigs.

The thermal death point for this organism is between 120° and 125°C. for 15 minutes.

Remarks. Morphologically this organism is similar to *Bacillus nondiasticus* described by Bergey. The organism reported by Bergey does not liquefy gelatin but reduces nitrates to nitrites and produces a slight acidity in milk. *Thermobacillus vulgaris* liquefies gelatin, does not reduce nitrates or alter litmus milk medium.

Thermobacillus digestans, nov. sp.

Four cultures of *Thermobacillus digestans*, nov. sp. type were isolated. This bacillus has rounded ends and granular protoplasm, with average dimensions of 5.6 by 0.8μ. The vegetative cell is Gram-negative and often forms short chains. The spores are oval and are located sub-terminally in the rod. Active motility.

An abundant, spreading, flat, glistening, and smooth growth was observed on plain agar slants. In nutrient broth a heavy pellicle was formed which settled to the bottom after 48 hours.

This organism fermented dextrose, maltose, and mannit with the production of acid but no gas. There were no changes in lactose, saccharose, salicin, starch, inulin, glycerin, or dulcite. Nitrates were reduced to nitrites in 24 hours and there was an evolution of free nitrogen gas. No hydrogen sulfide or indol was produced. In cooked meat medium this organism produced a slow but definite digestion, reducing the volume of meat particles by one half after 28 days' incubation, but without a foul odor. No amino acid crystals were formed after 28 days' incubation. In egg cube medium the albumen showed translucent areas after 2 days' incubation and was completely translucent after 7 days. This cannot be regarded as a true digestion, however, as the cube retained its original size and the edges were well defined throughout the entire period of observation.

In brain and alkaline egg no alteration in the medium was observed. Blood serum was partially liquefied, and after 48 hours a tube containing 30 cc. of gelatin was completely liquefied. Milk was coagulated with complete digestion after 7 days.

This culture produced no pathological changes when injected into a guinea pig.

The thermal death point lies between 145° and 150°C. for 15 minutes.

Remarks. No organism described by other investigators appears to have cultural reactions similar to those of this thermophile. MacFadyen and Blaxall (11) and Dupont (6) report the isolation of thermophilic bacteria which were proteolytic in character, but give no further description. *Thermobacillus digestans* is a true aerobic thermophile, which exhibits proteolytic activity.

Thermobacillus reductans, nov. sp.

Two cultures of the *Thermobacillus reductans*, nov. sp. type were isolated. This bacillus has rounded ends and a granular protoplasm, measures 3.0 μ in length by 0.6μ in width, and is Gram-positive and actively motile. The spores are terminal or subterminal and are oval.

The growth on plain agar slants was abundant, spreading, glistening, flat, and smooth. Nutrient broth cultures were cloudy, but there was no pellicle formation.

Dextrose, maltose, saccharose, salicin, and starch were fermented, with the production of acid but no gas. No change in the H-ion concentration was observed in lactose, inulin, glycerin, mannit, or dulcite. Nitrates were reduced to nitrites, but no hydrogen sulfide or indol was formed. There was no alteration in meat, egg cube, brain, or alkaline egg medium. Gelatin and Löffler's blood serum were slowly liquefied. Litmus milk was acidified and coagulated with a slowly progressing peptonization until the medium became translucent.

This organism was not pathogenic for guinea pigs.

The thermal death point for this culture lies between 135° and 140°C. at 15 minutes' exposure.

Remarks. Thermobacillus reductans is an aerobic thermophilic organism isolated from soil. In some few respects it corresponds to *Bacillus thermoindifferens*, described by Weinzirl (18). However, the organism described by Weinzirl does not reduce nitrates and grows equally well between 20° and 55°C. The culture described above reduces nitrates to nitrites and has a minimum temperature of growth at 40°C.

Thermobacillus restatus, nov. sp.

Three cultures of the *Thermobacillus restatus*, nov. sp. type, having the same morphology and exhibiting identical reactions, were isolated. This sporeforming bacillus averages 1.6 by 0.8μ in size and is Gram-positive and actively motile. The spores, when present, are oval and usually situated subterminally.

On plain agar slants the growth was abundant, spreading, flat, glistening, and smooth. No scum was formed in broth, but a veil-like sediment was found at the bottom of the tube.

There was no gas produced by this culture in carbohydrate media, but dextrose and maltose were fermented with the production of acid. Nitrates were not reduced and this culture did not break down peptone to produce indol. On lead acetate agar this organism showed a black growth, which indicated the production of hydrogen sulfide. In meat, egg cube, brain, alkaline egg, blood serum, gelatin, and milk media no change was observed.

This organism was not pathogenic for guinea pigs.

At 15 minutes' exposure the thermal death point of this organism lies between 150° and 155°C.

Remarks. Blau (4) isolated a thermophile, *Bacillus robustus*, which corresponds in some respects to *Thermobacillus restatus*. He fails, however, to observe the action on nitrate and several other media, and does not record the production of hydrogen sulfide. It is therefore impossible to make an exact comparison of *Bacillus robustus* and *Thermobacillus restatus* to establish their identity.

Thermobacillus ruber, nov. sp.

Thermobacillus ruber, nov. sp. has rounded ends and granular protoplasm, is Gram-positive, and the spores are oval and are situated at the end of the rod. It is actively motile and the average dimensions are 4.0 by 0.6μ.

In plain nutrient media this organism grew well. Acid but no gas was formed in dextrose, maltose, saccharose, salicin, starch, glycerin, and mannit. Lactose, inulin, and dulcite were not fermented. Nitrates were not reduced and there was no production of hydrogen sulfide or indol. In cooked meat and brain a deep pink color was produced but no further change. Blood serum was not liquefied, but a pink pigment was produced by the growth of this organism. No change in egg cube, alkaline egg, gelatin, or milk medium was noticed.

This organism was not pathogenic for guinea pigs.

The thermal death point for this organism for 15 minutes lies between 135° and 140°C.

Remarks. So far as the writer is aware, there is no thermophile described in the literature which is similar to this organism. It is true, however, that former workers did not employ the media on which *Thermobacillus ruber* produces its characteristic pink pigment. On plain agar or infusion agar this culture did not elaborate any coloration, but in meat, brain, and blood serum a definite pink pigment was formed within 48 hours.

Thermobacillus alcalinus, nov. sp.

Four cultures of the *Thermobacillus alcalinus*, nov. sp. type were isolated. The bacillus was Gram-negative, motile, with an average dimension of 6.4 by 0.8μ. The spores are round, located terminally and frequently subterminally.

The growth on plain agar slants was abundant, spreading, flat, glistening, and smooth. Nutrient broth was cloudy but no pellicle was produced.

Acid without gas was formed in dextrose, maltose, saccharose, salicin, starch, and mannit. Lactose, inulin, glycerin, and dulcite were not fermented. Nitrates were not reduced, gelatin and blood serum were not liquefied, and there was no production of hydrogen sulfide or indol. No change in meat, egg cube, brain, alkaline egg, or milk was observed.

This organism was not pathogenic for guinea pigs.

The thermal death point for a definite concentration of spores of this culture, exposed for 15 minutes, lies between 125° and 130°C.

Remarks. *Thermobacillus alcalinus* is a true aerobic thermophile isolated from soil. Weinzirl described an organism, which he called *Bacillus aerothermophilus*, that was in some respects similar to this organism. Weinzirl's organism did not reduce nitrates and fermented dextrose, sucrose, and mannitol, but litmus milk was reduced; while *Thermobacillus alcalinus* produced no change in this medium.

Thermobacillus violaceus, nov. sp.

Four cultures of the *Thermobacillus violaceus*, nov. sp. type were isolated. They were all Gram-negative, motile, spore-forming bacilli, averaging 3.0 by 0.8μ. The growth on agar slants was spreading, flat, glistening, and smooth. Growth in nutrient broth was characterized by cloudiness, present at the end of 12 hours.

Acid was formed in dextrose and saccharose media, but no gas was evolved. No fermentative action was detected on maltose, lactose, salicin, starch, inulin, glycerin, mannit, or dulcite. Nitrates were not reduced to nitrites and this culture produced no hydrogen sulfide or indol. Meat, egg cube, brain, and alkaline egg media were not altered by this culture. Gelatin was liquefied after 48 hours and blood serum showed a slight liquefaction after 7 days' incubation. Litmus milk was not coagulated, but the medium became deep purple, which was distinctly different from that of the control tube.

This organism was not pathogenic for guinea pigs.

The thermal death point of this culture was between 135° and 140°C. when exposed for 15 minutes.

Remarks. *Thermobacillus violaceus* is an aerobic thermophilic organism isolated from soil. In some few respects it corresponds to *Bacillus thermoalimentophilus*, described by Weinzirl. However, the organism isolated by Weinzirl reduced nitrates to nitrites and produced no acid in carbohydrate media.

Thermobacillus linearius, nov. sp.

Five cultures of the *Thermobacillus linearius*, nov. sp. type were isolated. The bacilli are actively motile, Gram-positive, appear singly in the smears, and average 4.0 by 0.8μ. Spores are round, usually terminal, and slightly greater in diameter than the sporangium, causing a bulging of the rod.

On plain agar slants the growth was heavy, spreading, glistening, opaque, and smooth. Broth cultures were cloudy, with a slightly mucoid sediment, but no pellicle was observed.

Acid was formed from dextrose, maltose, saccharose, salicin, inulin, glycerin, and mannit, while lactose, starch, and dulcite were not fermented. Nitrates were not reduced and there was no production of hydrogen sulfide or indol. No definite alteration of meat, egg cube, brain, or alkaline egg media was produced. Blood serum and gelatin were not liquefied and no change in milk was observed.

An actively growing broth culture of this organism was not pathogenic for guinea pigs.

The thermal death point for a definite concentration of spores for 15 minutes' exposure lies between 135° and 140°C.

Remarks. This organism is in some respects similar to *Bacillus thermotranslucens*, described by Bergey (2). The description of Bergey's organism is, however, not complete enough to identify this culture, since he does not include carbohydrate reactions, which are distinctive features of this species.

Thermobacillus catenatus, nov. sp.

The two cultures of *Thermobacillus catenatus*, nov. sp. that were isolated are bacilli with rounded ends and granular protoplasm. The vegetative cells are Gram-negative, motile, and measure 3.0μ in length by 0.8 in diameter. Spores, when present, are oval and terminally located.

On plain agar slants the growth of this organism was abundant, definitely spreading, flat, glistening, opaque, and smooth. Broth cultures were cloudy, with a thin, semitransparent film after 24 hours. Neither acid nor gas was formed from any of the carbohydrates used. Nitrates were not reduced. Hydrogen sulfide was produced, as indicated by a black growth on lead acetate agar. Indol was produced by this culture, but was only demonstrated by distilling off the indol, as suggested by Goré. Meat, egg cube, brain, and alkaline egg were not altered by this culture. Löffler's blood serum, after 4 days, was slowly liquefied, and gelatin, after 48 hours. Litmus milk was first coagulated and then slowly peptonized.

This culture was not pathogenic for guinea pigs.

The thermal death point of this culture lies between 135° and 140°C. when exposed for 15 minutes.

Remarks. This organism cannot be correlated with any description of thermophilic bacteria reported in the literature. Morrison and Tanner (14) reported the isolation of 52 cultures of thermophiles from water, all of which produced indol, but their descriptions are not complete enough to identify this culture.

DISCUSSION

The motility of this group of organisms presents some interesting considerations. It is generally believed that bacilli are motile through the lashing action of delicate, whiplike appendages called flagella. The sinuous or wormlike motility seen among the spirochaetes has never been observed in cultures of bacilli, and the active movement of a rod-shaped organism has come to be regarded as evidence of the presence of flagella. These delicate organs of locomotion do not take the ordinary dyes, and a variety of special staining methods have been devised for their demonstration. The technique employed is very exact, since the flagella are easily broken off from the cells and become lost in the surrounding medium. The methods of Löffler and Plimmer and Paine (16) were employed in attempts to stain any flagella present on the thermophiles studied in this investigation. Careful control of the technique was obtained by staining the peritrichic flagella on *Bacillus coli*. Both methods were equally successful, as the flagella of *Bacillus coli* were plainly seen under the microscope. When applied to the thermophiles under the same conditions, however, both methods failed to demonstrate flagella, either attached to the bacilli or broken off and free on the microscope slide. There may be some difference in the chemical composition of the flagella of thermophiles, because of their adaptation to growth at high temperatures, which makes the usual staining methods valueless. There is the possibility, however, that the motility of these organisms is not due to flagella but to some peculiar contractile property of the cytoplasm. Although the bacilli appeared rigid when examined in living preparations under the dark-field microscope, the internal arrangement of the cells could not be seen. When bacilli with flagella are examined under the dark-field, frequent peculiar flashes of light are caused by the rapid vibration of the flagella. This phenomenon was never observed in the examination of any of the thermophiles studied. The cause of motility in these cultures must, therefore, remain undetermined for the present.

MacFadyen and Blaxall (11) isolated from various sources several thermophilic bacteria, which grew at a temperature between 60° and 65°C.; their most marked property was the decomposition of protein bodies. Dupont isolated from manure a thermophilic bacterium which he described as being proteolytic in character. In this investigation *Thermobacillus digestans* shows a slow but definite proteolytic action. Gelatin and blood serum are liquefied, cooked meat medium is digested but no putrefactive odor is detected, and coagulated egg albumen is made translucent by the action of this organism.

Rettger (17), in his investigation on putrefaction, concludes that it takes place only under strictly anaerobic conditions, that it is always accompanied by the production of a foul odor, and that its end products are substances such as mercaptans. For example, *Bacillus sporogenes*, an obligate anaerobe,

when grown in cooked meat medium, alkaline egg, or egg cube medium, causes the dissolution of these substances, giving rise to foul-smelling gases. In contrast to this reaction, the apparent decomposition of the medium by aerobic bacteria is never accompanied by foul odors nor a reduction in the bulk of the protein substances included in the medium.

On the other hand, other investigators maintain that liquefaction of gelatin, blood serum, or of coagulated milk is an indication of proteolytic activity.

The action of *Thermobacillus digestans* on egg cube medium is also of particular interest. When incubated in this medium for 7 days at 55°C. the tube of coagulated albumen became perfectly translucent. This type of reaction suggested the possible presence of an extracellular enzyme liberated by the bacilli. Filtration experiments already described were negative, however, since the sterile filtrate of an actively growing culture in egg cube medium failed to produce translucency of the egg cube in the complete absence of the bacteria. It seems evident that this reaction takes place only when the culture is growing in the medium. A similar reaction on this medium is produced by certain anaerobes. Thus *Bacillus botulinus*, after 3 days' incubation at 37°C., produces gas in egg cube medium, but the cube is two-thirds digested, whereas the remaining portion is rather translucent. Likewise the anaerobes *Bacillus putrificus* and *Bacillus bifermentans*, as described by Kahn (10), produce a translucency of the egg cube, but there is also a digestion, as indicated by the fact that the edge becomes scalloped. *Thermophilus digestans* produces a complete translucency of the egg cube, but even after 28 days' incubation at 55°C. the edges are well defined and there is no indication of digestion.

CONCLUSIONS

1. The biological and cultural activities of ten aerobic, spore-forming, motile, obligate thermophiles are described.

2. The optimum temperature for growth falls between 50° and 60°C. Growth does not take place below 40°C. and ceases when the temperature is raised to 80°C.

3. The thermal death point for a definite concentration of spores varies with each species. The limits lie between 125° and 155°C. for 15 minutes.

4. One aerobic obligate thermophile is described which exhibits proteolytic action.

5. The cultures are not pathogenic for guinea pigs.

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MINNESOTA GLACIAL SOIL STUDIES: II. THE FOREST FLOOR ON THE LATE WISCONSIN DRIFT[1]

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INTRODUCTION

In studies of the soils of American forests, little attention usually has been devoted to the forest floor, or to the part of this known as leafmold, as distinct from the immediately underlying five or six inches of soil. This is because most of such soil studies have been made from a strictly agricultural viewpoint and not from that of either forestry or soil science. Where the timber has been cut off, and the land used as a pasture for some time before removing the stumps, the layer of leafmold has usually become thin and firmly bound by the grass roots to the mineral matter below. When the sod is plowed, whether or not this has been preceded by the removal of all the stumps, the leafmold is turned under and whatever plant nutrients and soil ameliorating materials it carries become integral parts of the surface soil. Even if the original forest trees still remain at the time of sampling, or if there should be a second growth of trees and brush, a great change in the forest floor may take place before the land is plowed.

The term *forest floor* is here used "to designate only the deposits of vegetable matter on the ground in a forest" as proposed in 1917 by a committee on forest terminology of the Society of American Foresters and of the Canadian Society of Forest Engineers (2, p. 78) which further proposed three subdivisions of the forest floor:

1. *Litter*—the upper, but slightly decomposed, portion of the forest floor;
2. *Leafmold*, or *humus*—the portion in which decomposition of the litter is so far advanced that its original form is not distinguishable (2, p. 82);
3. *Duff*—an intermediate layer of more or less decomposed organic matter between the litter and the leafmold.

The term *humus*, already so variously used by soils investigators, does not appear to the authors as satisfactory as the already well established synonym —*leafmold* (2, p. 85).

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The data presented in this paper were secured in a study of virgin forest soils compared with virgin prairie soils of the same age, both developed on the same calcareous glacial drift—the till plains of the Late Wisconsin glaciation. In this, the authors took extreme precautions, both to select for sampling only those woods which were most nearly virgin and to separate sharply the leafmold layer from the underlying mineral soil, the first foot of which was sampled in three-inch sections.

With the more or less disintegrated plant debris were included whatever fallen leaves and small woody fragments were present, but all living plants that might be within the selected squares were rejected. Thus the term *forest floor* is used in the narrow sense of "Waldstreu," as defined by Ramann

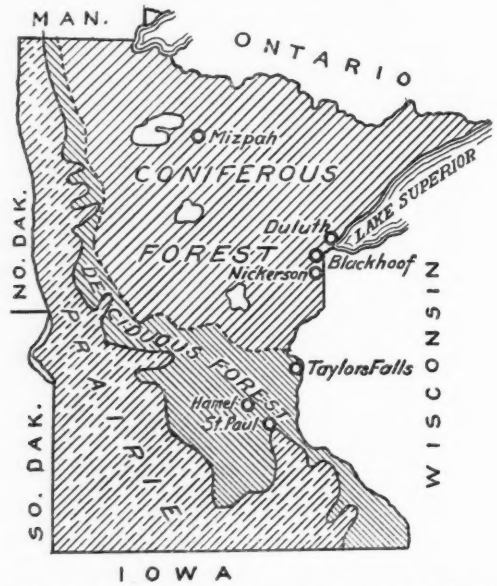

FIG. 1. MAP OF MINNESOTA SHOWING LOCATIONS OF WOODS SAMPLED

(5, p. 462), and not in its wider sense, which would include the soil cover and accordingly the low-growing plants which occur under the protection of the forest.

The samples were collected in August and September, 1916, from nine wooded fields; three near Hamel, three near Taylors Falls, and three near Mizpah (table 1). The fields of the first group lie about 20 miles west of St. Paul and remain from the deciduous forest known as the Big Woods of Minnesota. Those of the second group are about 40 miles northeast of St. Paul, lying at the eastern edge of the state, and form remnants of a forest very similar to the Big Woods. The Mizpah group is about 200 miles north of St. Paul and well within the coniferous forest (fig. 1).

TABLE 1

Location of woods sampled

GROUP	FIELD NUMBER	RANGE, WEST	TOWNSHIP, NORTH	SECTION	PART OF SECTION
Hamel	I	23	118	2	SE¼ of NE¼
	II	23	119	16	NE¼ of SE¼
	III	24	119	25	SE¼ of NW¼
Taylors Falls	I	19	34	22	SW¼ of SE¼
	II	19	34	10	SW¼ of SW¼
	III	19	35	31	NW¼ of NW¼
Mizpah	I	27	152	30	NE¼
	II	27	152	10	NE¼
	III	28	151	10	E½

TABLE 2

Climatological data

GROUP	APPROXIMATE ELEVATION	LENGTH OF RECORD	LENGTH OF GROWING SEASON	MEAN TEMPERATURE					SNOWFALL	PRECIPITATION
				Winter	Spring	Summer	Fall	Year		
	feet	*years*	*days*	*°F.*	*°F.*	*°F.*	*°F.*	*°F.*	*inches*	*inches*
Hamel*	1,000	34	150	16	44	70	48	45	44	28.39
Taylors Falls†	950§	34	140	12	42	67	45	42	52	31.46
Mizpah‡	1,400	38	100	8	38	65	42	38	50	25.33

* Record from Minneapolis, 13 miles distant.

† Record from Grantsburg, Wis., 30 miles distant.

‡ Record from Winnibigoshish, 35 miles distant.

§ Approximate elevation of woods and not of the U. S. Weather Bureau station, which is in the St. Croix Valley and only 759 feet above sea level.

At Hamel and Taylors Falls the climate is much alike, whereas at Mizpah both the temperature and rainfall are lower, the growing season is much shorter and the duration of the snow cover is much longer (table 2).

CHARACTER OF FOREST GROWTH

The woods at Hamel and Taylors Falls either belong to the maple-basswood type of forest or form a transition from this to the oak-maple type, whereas those at Mizpah belong to the spruce, balsam-birch type (4).

Hamel group

Of the Hamel group, I was the most nearly virgin, only a few trees having been removed from it. Hard maples (*Acer saccharum*) were the most nu-

merous trees, with some red oak (*Quercus rubra*), bur oak (*Quercus macrocarpa*), and basswood (*Tilia americana*) and a few elm (*Ulmus americana*) and ash. Considerable undergrowth of young maples and a few ferns were present but there was no grass. Field II, five miles northwest of the preceding, showed more alteration; scattered tufts of grass were present but these were avoided in sampling. Hard maples and elms were most numerous, with some basswood, red oak, and a very few butternut trees (*Juglans cinerea*). There was about as much undergrowth as in I, consisting of young maples and elms with a few ferns. From III, which lay to the west of the others and about 5 miles from each, still more trees had been removed. Although somewhat more grass was present, the most of the forest floor showed none. Samples were taken only from grass-free sites. About 90 per cent of the trees were hard maples, with elms next in number. The general character of the interior of the Hamel woods may be seen from plate 1, showing II and III.

Taylors Falls group

The most nearly virgin wood in the Taylors Falls group was I, in which basswoods predominated, with many butternuts and smaller numbers of bur oak, white birch (*Betula papyrifera*), and poplar (*Populus tremuloides*). Many small elms were present and ferns were numerous. Wood III appeared almost as nearly virgin as I; very little grass was observed and only three large stumps were found in the six acres. About 80 per cent of the trees were hard maples and the remainder basswood, except for a few elms and butternuts and a very few large white oaks (*Quercus alba*). Ferns were abundant (plate 1). More grass and large stumps were found in II than in the others. In it also the trees were mostly hard maples, with a considerable number of basswood and a few white birch; the undergrowth included ferns and many young elms.

Mizpah group

In the Mizpah group the conifers predominated. Field I was occupied by balsam (*Abies balsamea*) and spruce, with a few white birch; no trees had been cut. From II, in which white cedars (*Thuja occidentalis*) were most numerous along with fewer balsam and some white birch, some of the larger cedars had been removed. In III the trees were chiefly balsam, many of them small, with a few spruce, white birch, white cedar, and balsam poplar (*Populus balsamifera*). The forest floor was thicker in this wood than in any of the eight others.

METHODS OF SAMPLING AND ANALYSIS

As criteria of the degree of virginity of the forest, in selecting the woods for sampling, the forest floor, the amount of grass, the presence of undergrowth, evidences of forest fires in the past, and the extent to which trees had been

removed, as indicated by the stumps, were considered. After a wood had been definitely decided upon, a level uniform area within it was chosen for the actual sampling.

In order to determine the weight of the forest floor per unit area and to secure samples for analysis, a square wooden form similar to an ordinary picture frame, with inside dimensions of exactly 12 by 12 inches was used. This was placed on the ground and any living plants within it were cut off and thrown away. Then with a sharp knife, a square foot section of the floor was carefully separated from the underlying mineral soil and placed in a sack. From 5 to 10 such samples were taken in each wood, the exact number depending upon the uniformity in thickness of the forest floor. The composite sample was well mixed and weighed, and a weighed portion of it saved for a moisture determination at 100°C. The rest was later dried and thoroughly ground before being used in the laboratory studies.

In the case of each sample the authors determined the moisture equivalent at 1000 times gravity, nitrogen by the Kjeldahl-Gunning method, volatile matter by prolonged ignition at dull redness and acidity by the Truog method. For the organic carbon and phosphoric acid, composites prepared from equal weights of the samples from the three woods in each group were used. The organic carbon was determined by mixing the dried material with copper oxide and burning in a current of oxygen, and the phosphoric acid by igniting, extracting the resulting ash with aqua regia and using this extract for precipitation.

For comparison with the forest floor, tables 3, 4, and 5 show some data on the underlying 6 inches of soil. In all the woods this was sampled in two sections 1 to 3 and 4 to 6 inches. In the Hamel and Taylors Falls groups the density of each of the two sections was determined (3). In arriving at the values for the 6 inches, such data as the amount of nitrogen and of volatile matter in each of the two sections have been computed separately and the sum of the two used instead of multiplying the average weight per acre—6 inches by the average percentage of nitrogen or of volatile matter. This is necessary in order to secure correct values, because the first 3-inch section is much less dense than the second while much richer in nitrogen and organic matter.

In the computations of the data for Mizpah woods, in which no density determinations were made, the averages of the densities found for the first and second sections in the Hamel and Taylors Falls woods were used.

AMOUNT AND CHARACTER OF FOREST FLOOR

Amount

The quantity per unit area varies greatly from wood to wood, at Hamel from 0.58 to 1.28 pounds of oven-dry material per square foot, at Taylors Falls from 0.90 to 0.96, and at Mizpah from 1.07 to 4.44. The first is equiva-

TABLE 3

Amount, properties, and composition of forest floor compared with underlying 6 inches of soil

GROUP	FOREST FLOOR				SURFACE 6 INCHES OF SOIL			
	Wood I	Wood II	Wood III	Average	Wood I	Wood II	Wood III	Average
Weight per square foot of dry material, in pounds								
Hamel	1.15	1.28	0.58	1.00	31.3	36.2	34.6	34.0
Taylors Falls	0.93	0.96	0.90	0.93	32.3	32.4	34.9	33.2
Mizpah	1.39	1.07	4.44	2.30	.....	.....	.....	.....
Color, when moistened to the moisture equivalent								
Hamel	Dark grayish brown	Brown	Grayish brown	.....	Grayish black	Dark gray	Dark gray	.....
Taylors Falls	Reddish brown	Light brown	Brown	.....	Dark gray	Dark gray	Dark gray	.....
Mizpah	Reddish brown	Reddish brown	Dark reddish brown	.....	Gray	Very light gray	Dark gray	.....
Acidity, determined by Truog method								
Hamel	None	None	None	.....	Slight	Slight	Slight	.....
Taylors Falls	None	None	None	.....	Slight	Slight	Slight	.....
Mizpah	Slight	Slight	Slight	.....	Very slight	Slight	None	.....

Volatile matter, in per cent

Hamel	51.8	57.3	62.6	57.2	.....	.....	.....	5.99
Taylors Falls	62.2	63.8	62.3	62.8	.....	.....	.....	5.37
Mizpah	80.7	76.4	61.6	72.9	.....	.....	.....	2.77

Moisture equivalent

Hamel	97.9	111.1	97.0	102.0	26.0	24.5	20.7	23.7
Taylors Falls	112.8	111.4	112.6	112.3	19.4	20.8	21.2	20.5
Mizpah	119.4	120.2	108.8	116.1	17.7	13.8	17.4	16.3

Nitrogen, in per cent

Hamel	1.53	1.57	1.76	1.62	0.232	0.190	0.173	0.198
Taylors Falls	1.86	1.74	1.82	1.81	0.182	0.192	0.160	0.178
Mizpah	1.89	1.55	1.47	1.64	0.071	0.046	0.063	0.060

Nitrogen in volatile matter, in per cent

Hamel	2.95	2.74	2.81	2.83	.....	.....	.....	3.30
Taylors Falls	2.99	2.73	2.92	2.87	.....	.....	.....	3.31
Mizpah	2.34	2.02	2.39	2.25	.....	.....	.....	2.16

TABLE 4

Weight of forest floor and various constituents

Pounds per acre

	WOOD I	WOOD II	WOOD III	AVERAGE
Weight of dry forest floor				
Hamel	50,094	55,757	25,265	43,705
Taylors Falls	40,511	41,818	39,204	40,511
Mizpah	60,548	46,609	193,406	100,188
Volatile matter				
Hamel	25,949	31,948	15,816	24,200
Taylors Falls	25,198	26,680	24,424	25,434
Mizpah	48,862	35,609	119,138	67,870
Nitrogen				
Hamel	765	878	444	696
Taylors Falls	752	727	713	731
Mizpah	1,145	722	2,847	1,571
Nitrogen in surface 6 inches of soil				
Hamel	2,823	2,730	2,319	2,624
Taylors Falls	2,104	2,265	2,141	2,170
Mizpah	913	613	828	785
Nitrogen in forest floor and surface 6 inches of soil together				
Hamel	3,588	3,608	2,763	3,320
Taylors Falls	2,856	2,992	2,854	2,901
Mizpah	2,057	1,335	3,675	2,356
Proportion of above nitrogen carried in the forest floor, expressed as per cent				
Hamel	21.3	24.3	16.1	20.6
Taylors Falls	26.3	24.3	25.0	25.2
Mizpah	55.6	54.1	77.5	62.4

Phosphoric acid

	HAMEL	TAYLORS FALLS	MIZPAH
a. In forest floor	135	125	304
b. In surface 6 inches of soil*	1799	1853	999
c. In forest floor and surface soil	1934	1978	1303
d. Percentage of whole present in forest floor	6.9	6.3	23.3

* Total amount as determined by ordinary rock analysis.

lent to 12.6 tons and the last to 96.7 tons per acre. The average for the Hamel and Taylors Falls woods is 21.0 tons per acre. Although the average is higher for Mizpah than for either of the other groups, there was less forest litter in II at Mizpah than in either I or II at Hamel, the high average being due to the very thick floor in III.

Color

The forest floor showed a brownish color at all three places but with a reddish tint at Mizpah and a gray one at Hamel.

TABLE 5

Organic carbon and phosphoric acid in the forest floor and the underlying 6 inches of soil

	HAMEL	TAYLORS FALLS	MIZPAH
Organic carbon:			
a. In forest floor.....*per cent*	30.77	33.15	39.47
b. In surface soil.....*per cent*	2.66	2.25	0.85
Organic matter, computed (organic C $\times$ 1.724):			
a. In forest floor.....*per cent*	53.10	57.10	68.10
b. In surface soil.....*per cent*	4.58	3.88	1.46
Ratio of volatile matter to organic carbon:			
a. In forest floor.....	1.86	1.89	1.85
b. In surface soil.....	2.25	2.39	3.26
Ratio of organic carbon to nitrogen:			
a. In forest floor.....	19.0	18.4	24.1
b. In surface soil.....	13.1	12.3	13.5
Ratio of volatile matter to computed organic matter:			
a. In forest floor.....	1.1	1.1	1.1
b. In surface soil.....	1.3	1.4	1.9
Phosphoric acid (P_2O_5):			
a. In forest floor.....*per cent*	0.309	0.309	0.303
b. In surface soil.....*per cent*	0.125	0.140	0.060
Ratio of organic carbon to phosphoric acid:			
a. In forest floor.....	100	107.0	130.0
b. In surface soil.....	21	16.0	14.0

Acidity

The Hamel and Taylors Falls floors showed no acidity and those from Mizpah but little. However, it should be pointed out that all were from woods in which the underlying surface soil showed very little acidity (table 3).

Volatile matter

The volatile matter in the samples, as collected, varied from 51.8 to 80.7 per cent, with an average of 57 at Hamel, 63 at Taylors Falls, and 73 at Mizpah. It is of interest that the sample from the Mizpah wood that showed

much the greatest weight per unit area also carried much the highest proportion of mineral matter. This provides a good illustration of the importance of determining the volatile matter, as well as the dry weight per unit area, in order to compare the amounts of organic matter found in different woods or reported by different investigators. When one, in attempting to include the last trace of leaf mold, scrapes off the mineral soil very closely, he is apt to include so much of the latter that the proportion of volatile matter will be appreciably lowered.

On account of the influence of the included mineral soil upon the composition of the samples—the proportion of this differing with different workers and from time to time—it is desirable to express the percentages and weights on the basis of the volatile portion as well as of the whole of the forest floor.

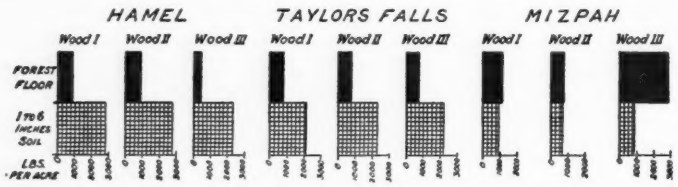

FIG. 2. AMOUNT OF NITROGEN PER ACRE IN THE FOREST FLOOR AND IN THE UNDERLYING 6 INCHES OF SOIL

Nitrogen

Nitrogen varied from 1.47 to 1.89 per cent, both extremes being found in the Mizpah group. Computed for the volatile portion, the nitrogen varies from 2.02 to 2.99 per cent, averaging much alike at Hamel and Taylors Falls—2.83 compared with 2.87 per cent—but being considerably lower in the Mizpah woods—varying from 2.02 to 2.39 per cent. In the surface 6 inches of soil the percentage of nitrogen is only from one-seventh to one-thirty-fourth as high as in the overlying forest floor.

The amount per acre of nitrogen in the floor varies from 444 pounds in III at Hamel to 2847 in III at Mizpah (fig. 2). Although the highest two values occur at Mizpah and the lowest at Hamel, the other six are very similar. A corresponding variation is to be observed in the amounts per acre of volatile matter. When the total amount of nitrogen contained in the floor and in the surface 6 inches of soil is considered, it is seen that in the deciduous woods the former carries only from 16 to 26 per cent but in the coniferous forest from 54 to 77 per cent. Accordingly the complete burning of the forest floor in one of the southern woods would liberate only about one-fifth of the total amount of nitrogen present, but in the northern forest, half or more, thus making the loss far more serious, provided that the nitrogen in both were at once, or eventually became, equally available, an assumption unproved but still quite probable.

Organic carbon

Organic carbon is highest—39.47 per cent— in the floor at Mizpah, and lowest—30.77—in that from Hamel, although the average nitrogen content in these two groups is practically alike. The organic matter, computed from the organic carbon, amounts to 68.1 per cent in the Mizpah litter, compared with only 53.1 at Hamel. The weight per acre of organic matter, computed from the organic carbon, is about one-eleventh less than the weight of volatile matter. This difference is probably due to the presence of so-called *water of constitution*, which is driven off while the organic matter is undergoing oxidation, and may be due in part to the factor used—1.724—being too low. This difference between the computed organic matter and the determined volatile matter is not serious, but much more weight should be attached to the former than to the latter.

Ratio of organic carbon to nitrogen

In the surface 6 inches of soil in these virgin woods, the ratio of organic carbon to nitrogen varies only between narrow limits, averaging 13.0. It is much higher, however, in the overlying floor, varying from 18 to 24 in the three groups of woods.

Phosphoric acid

In phosphoric acid content all three group composites are alike, carrying 0.30 to 0.31 per cent. At Hamel and Taylors Falls the weight of the phosphoric acid carried in the floor, being only about one-sixteenth of the total, is low compared with that in the underlying soil, but at Mizpah it forms about one-fourth. The effect of burning off the floor may be very marked on soils poorly supplied with readily available phosphoric acid, especially with crops responsive to phosphate applications, for the phosphate left behind will be largely in a form readily available for plants.

Moisture equivalent

The moisture equivalent is high for all the samples of floor, with a maximum of 120.2, a minimum of 97.0, and an average of 110.1, contrasted with 26.0, 13.8, and 20.2 for the underlying surface 6 inches of soil. Assuming that the water-retaining capacity of both floor and soil is equal to the moisture equivalent (1, p. 65), it is evident that after a rain the floor may retain a very large amount of water—almost one inch of rain (0.93 inch) in wood III at Mizpah but only about 0.10 inch in III at Hamel—before allowing any to pass into the underlying soil. As in the forest the moisture content of the floor is never reduced to zero, the amounts of rain which even in its driest condition it could absorb would be considerably below these values.

There is no close connection observable between the percentage of volatile matter in the floor and its moisture equivalent. The samples with the highest

percentages of volatile matter (Mizpah I and II) have the highest moisture equivalents (119.4 and 120.2), and the three at Taylors Falls with similar volatile matter content (62.2 to 63.8 per cent) have similar moisture equivalents (111.4 to 112.8). The sample from III at Hamel, with 62.6 per cent volatile matter, however, has a moisture equivalent of only 97.0, and I at Hamel, with a similar moisture equivalent (97.9) carries only 51.8 per cent volatile matter.

AN EARLIER STUDY

In table 6 are reported data on three samples of forest floor and of the underlying 6 inches of soil collected near Duluth in September, 1915. The one was from virgin coniferous forest on the Duluth Experimental Farm near the city of Duluth, the soil of which is a stony clay loam developed on the Superior lobe of the Late Wisconsin glaciation (6, p. 164–165). Three years later this forest, consisting chiefly of balsam (*Abies balsamea*), was destroyed by the disastrous forest fire of October 12, 1918.

TABLE 6

Forest floor of woods near Duluth

	DULUTH	BLACKHOOF	NICKERSON
Forest floor:			
Nitrogen, *per cent*	1.48	1.25	1.56
Organic carbon, *per cent*	39.14	36.16	40.76
Carbon-nitrogen ratio	26.4	29.0	26.1
Weight per square foot, *pounds*	1.56	.....	.....
Moisture equivalent	127.8	122.8	137.7
1 to 6 inches of soil underlying forest floor:			
Nitrogen, *per cent*	0.17	0.19	0.05
Organic carbon, *per cent*	2.88	2.49	0.95
Carbon-nitrogen ratio	17	13	19
Weight per square foot, *pounds*	33.0	.....	.....
Moisture equivalent	27.1	34.9	6.4

The site at Blackhoof is 27 miles southwest of Duluth on Superior clay, developed on the lacustral material laid down in glacial Lake Duluth. The forest had originally consisted of a mixture of hardwoods and white pine (*Pinus strobus*), but the latter had been removed some years before the time of sampling.

The third site, that at Nickerson, was 8 miles south of the preceding on a very sandy moraine near the south shore of glacial Lake Duluth. It was covered with jack pine (*Pinus Banksiana*) and red pine (*Pinus resinosa*).

The methods of sampling and of analysis were similar to those described above. The samples are similar in composition and properties to those reported in tables 3, 4, and 5.

SUMMARY AND CONCLUSION

The forest floor was sampled in 9 virgin or nearly virgin Minnesota woods, 6 being remnants of the original southeastern deciduous forest and 3 being in the northern coniferous forest.

The amount per acre was found to vary from 13 to 97 tons, and the content of nitrogen from 1.47 to 1.89 per cent, of volatile matter from 52 to 81 per cent, corresponding to 444 to 2,847 pounds of nitrogen and 8 to 60 tons of volatile matter per acre. The nitrogen in the volatile matter varied from 2.02 to 2.99 per cent, the organic carbon from 31 to 39 per cent, and the ratio of organic carbon to nitrogen from 18 to 24. The samples showed little or no acidity, carried about 0.30 per cent phosphoric acid, and had moisture equivalents of 97 to 120.

A comparison of the weight per acre of nitrogen in the forest floor with that in the underlying 6 inches of soil, showed the one extreme to be in a hard maple wood in which the floor carried only one-sixth as much as the surface soil, and the other in a spruce-balsam-birch forest, in which the floor carried more than three times as much nitrogen as the surface 6 inches of soil.

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PLATE 1

ILLUSTRATIONS SHOWING CHARACTER OF WOODS AT HAMEL AND TAYLORS FALLS

FIG. 1. Wood II of the Hamel group. The cordwood, shown in the foreground, had been cut between the time of sampling and that of taking the photograph, in September, 1919.

FIG. 2. Interior of Wood III at Taylors Falls, with dense growth of ferns in foreground.

FIG. 3. Interior of Wood III at Hamel.

FIG. 1

FIG. 2

FIG. 3

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MINNESOTA GLACIAL SOIL STUDIES: III. DENSITY OF THE SURFACE FOOT IN FOREST AND PRAIRIE ON THE LATE WISCONSIN DRIFT[1]

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INTRODUCTION

In connection with a study of the composition of virgin soils developed upon the till plains of the Late Wisconsin glaciation, the density was determined for each of the four 3-inch sections of the surface foot in six woods and in six prairie fields—remnants of the original prairies. Only where information is available on the density as well as on the composition of the virgin soils is it possible to draw reliable conclusions as to the effect of cultivation on the organic matter and nitrogen content. Comparatively few data on the density of surface soils *in situ* have been reported and most of these have dealt with sections of 6 to 12 inches from fields that were far from virgin. Studies on the relative density of different depths of the surface soil have been reported by Alway and his co-workers (2; 3, p. 221–226; 4).

According to Hilgard (5, p. 107–8) "the loose tilth and humus content of the surface soil will in general cause it to weigh less, bulk for bulk, than the underlying subsoil, even when the latter is more clayey; moreover, the continous pressure from above will tend to consolidate the subsoil and substrata;" and sandy land may be assumed to have a weight per cubic foot of about 92 pounds, loams, 80, clay land 75, and garden land and woods-earth 69 pounds.

Warington reports the density of two fields at Rothamsted and one at Woburn (table 1) and mentions that although the density increased with depth to 36 inches, samplings made to a greater depth showed little further increase. That the surface 9 inches of the old pasture was much lighter, and the lower three sections heavier than those from the arable land, he considered "an excellent illustration of the lightening of a soil laid down to grass, brought about chiefly by the accumulation of vegetable residues in the surface soil" (7, p. 46).

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DESCRIPTION OF FIELDS

Twelve fields with virgin soil, forming four groups of three each, were included in this investigation. The groups are located approximately in an east and west line across the state, two on the prairie and two in the deciduous forest, and all on the unmodified till plain of the Late Gray Drift—the Des Moines lobe of the Late Wisconsin glaciation. The Taylors Falls fields are only a few miles from the Wisconsin boundary, the Canby fields are close to the South Dakota line, and the other two groups lie between.

All the Canby and Renville fields were still in prairie at the time of the study—1916—and virgin to the extent that the native sod had never been disturbed nor the fields fenced nor closely pastured, although for a number of years the crop of native grasses had been removed as hay. Their soils were typical of the prairie—black or brownish black, and high in content of organic matter. The composition of these soils will be dealt with in detail in a later paper of this series. The Taylors Falls and Hamel fields, occupied by deciduous forest, have been described in the preceding paper (2).

TABLE 1

Density of three English soils, from data of Warington

Weight per cubic foot

DEPTH	ROTHAMSTED HEAVY LOAM, OLD PASTURE	ROTHAMSTED HEAVY LOAM, ARABLE LAND	WOBURN LIGHT SAND, ARABLE LAND
inches	*pounds*	*pounds*	*pounds*
1–9	71	89	97
10–18	95	93	104
19–27	100	98	106
28–36	102	101	107

After a field had been decided upon, two sites, on a level uniform area within it, both well removed from large trees, were selected and from these the density samples were taken. The apparatus consisted of a box of heavy reinforced sheet iron, 12 inches long, 6 inches wide, and 12 inches high, inside measurements, without top or bottom. It was made up of four sections, placed one above the other, each 3 inches deep, held together by two bolts passing vertically through small extensions at the ends of each section. These with the aid of thumb nuts held the four sections firmly together. It was found important to have the inner surface as smooth as possible, and to have each section fit squarely against the adjoining sections.

Around the block of soil selected for the density determination a trench was dug 15 inches deep and wide enough to allow free handling of a spade. The block at the beginning measured about 8 by 14 inches horizontally. The apparatus was placed on top and the block trimmed, by means of a large sharp knife, just enough to permit the apparatus to be pressed down over it. Care was necessary in trimming the block to prevent any bulging of the box, while

avoiding all unnecessary trimming. The work was facilitated by the presence of a fair supply of moisture in the soil at the time and by the small number of stones and tree roots. The latter passing through a block of soil presented the most difficult obstacle. Generally the roots encountered were small, although a few an inch or two in diameter were found. Where a root was met with after a block had been partly removed, the apparatus was worked down close to it and the root then trimmed with a very sharp hatchet, just enough to permit it to pass within the box. When a stone protruded from the block, or occupied parts of two adjacent sections, it was removed and weighed, care being taken to estimate the volume of the portion in each section. The proportionate weights were then assigned to the proper sections.

When the box had been lowered until the top was flush with the surface of the mineral soil, the forest floor having been removed, the block was cut from its base, the box tipped onto its side, and the contents were removed from the pit to an oilcloth. Wherever the surface was dry and crumbly, a sheet-iron lid was placed on the top to prevent loss. The lower, irregular surface was supported by one hand, and, as soon as the block had been placed upon its side on the oilcloth, the bottom was trimmed flush with the lower edge of the box and the trimmings were thrown away. A second sheet of oilcloth was then brought under one end of the box, the nuts were loosened, the bolts were withdrawn until the first section was released, and a large, long straight-edge knife was inserted between it and the second. It was found better to cut slightly into the section being removed, rather than squarely along the edge of it. When the soil section had been separated from the rest of the block and its box removed, the surface of the adjacent section was then trimmed flush with the edge of its box and the trimmings were added to the soil from the first section. The question of whether to remove the 1 to 3-inch or the 10 to 12-inch section first was determined by the moisture content, texture, and other conditions of the soil, the looser end being dealt with first. In the prairie fields, as the surface sections were generally well bound together by roots, the 10 to 12-inch section was removed first. In the forest fields it was found better to remove the 1 to 3-inch section first, the lowest being more compact and generally more moist and holding together much better. After the whole of a 3-inch section had been transferred to the second oilcloth, it was carefully mixed and weighed, and a weighed portion was taken and placed in a sack to be sent to the laboratory for a moisture determination. A portion of the remainder was saved for the preparation of a composite sample for chemical analysis. Then the oilcloth was cleaned, placed under the second soil section, and the procedure repeated.

For the laboratory determination, the composite sample was prepared by mixing equal volumes from the corresponding sections from the two sites in a field. The density data reported for each field are the averages from the two sites.

THE DENSITY

The density, expressed as the weight per cubic foot, is reported in table 2. In the comparison of the successive sections from the same field the most striking difference appears in the forest fields, where the surface is much less dense than the sections below. There is a similar but not nearly so marked difference in the prairie fields, as may be seen better from table 3, which gives for

TABLE 2
Density of the different sections
Weight per cubic foot

DEPTH	FIELD I	FIELD II	FIELD III	FIELD I	FIELD II	FIELD III
inches	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
	Canby			*Renville*		
1–3	55.5	56.2	60.1	46.5	52.4	58.3
4–6	63.9	72.9	69.9	62.5	52.4	62.3
7–9	69.0	79.4	75.8	69.0	53.2	63.5
10–12	71.1	82.9	81.8	71.5	60.4	66.4
Average 1–12	64.9	72.8	71.9	62.4	54.6	62.6
	Hamel			*Taylors Falls*		
1–3	48.4	62.2	57.9	45.0	49.7	55.0
4–6	77.0	82.8	80.5	84.5	79.9	84.6
7–9	85.9	93.9	93.4	87.5	90.4	95.7
10–12	85.8	96.5	96.6	97.4	95.2	101.1
Average 1–12	74.3	83.8	82.1	78.6	78.8	84.1

TABLE 3
Relative density of the four sections
The first taken as unity

DEPTH	FIELD I	FIELD II	FIELD III	AVERAGE	FIELD I	FIELD II	FIELD III	AVERAGE
inches								
	Canby				*Renville*			
1–3	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
4–6	1.2	1.3	1.2	1.2	1.3	1.0	1.1	1.1
7–9	1.2	1.4	1.3	1.3	1.5	1.0	1.1	1.2
10–12	1.3	1.5	1.4	1.4	1.5	1.2	1.2	1.3
	Hamel				*Taylors Falls*			
1–3	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
4–6	1.6	1.3	1.4	1.4	1.9	1.6	1.5	1.7
7–9	1.8	1.5	1.6	1.6	1.9	1.8	1.7	1.8
10–12	1.8	1.6	1.7	1.7	2.2	1.9	1.8	2.0

each field the ratio of the density of each of the lower sections to that of the surface, which is taken as unity. In all the fields the density increases, in general, with each increase in depth, from which it may safely be concluded that if successive sections had been taken from a large number of sites in each

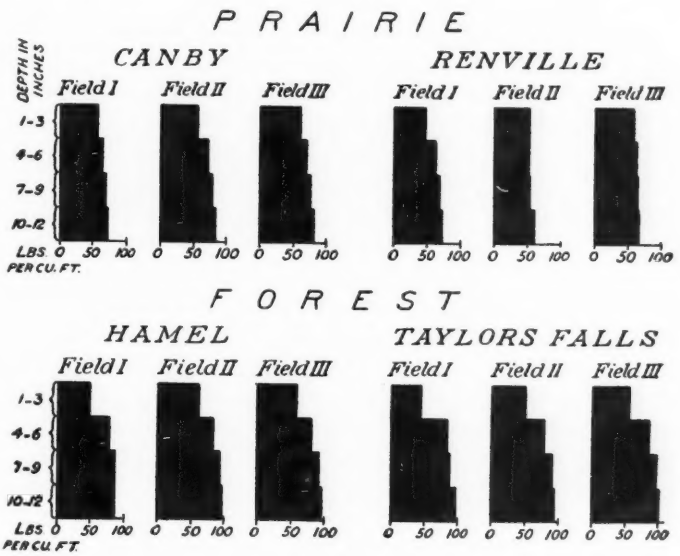

FIG. 1. DENSITIES OF THE FOUR SECTIONS OF THE TWELVE FIELDS

TABLE 4

Density of the different sections of the four groups of fields

Weight per cubic foot

DEPTH	FOREST FIELDS		PRAIRIE FIELDS		AVERAGE FOR FOREST FIELDS	AVERAGE FOR PRAIRIE FIELDS	AVERAGE FOR ALL FIELDS
	Taylors Falls	Hamel	Renville	Canby			
inches	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
1–3	49.9	56.2	52.4	57.3	53.0	54.8	53.9
4–6	83.0	80.1	59.0	68.9	81.6	64.0	72.8
7–9	91.3	91.1	61.9	74.8	91.1	68.3	79.7
10–12	97.9	93.0	66.1	78.6	95.4	72.3	83.8
1–12	80.5	80.1	59.9	69.9	80.3	64.9	72.6

field, instead of from only two, an increase with depth would have been found in all the fields. In the forest the increase is more marked than on the prairies.

When the fields of the various groups are considered separately, the fields of each group show considerable uniformity (fig. 1). The lower densities

of Field I at Canby, Field II at Renville, and Field I at Hamel are associated with a higher organic matter content.

The average densities for the different groups are given in table 4. In the surface section there is no distinct difference between prairie and forest.

TABLE 5

Moisture equivalents of the soils of the different fields

DEPTH	FIELD I	FIELD II	FIELD III	AVERAGE	FIELD I	FIELD II	FIELD III	AVERAGE	AVERAGE
inches									
	Canby				*Renville*				*Prairie Fields*
1–3	36	30	27	31	39	39	36	38	34
4–6	31	28	26	28	33	34	33	33	31
7–9	29	26	25	27	30	33	31	31	29
10–12	28	25	25	26	28	31	30	30	28
	Hamel				*Taylors Falls*				*Forest Fields*
1–3	30	28	25	28	23	25	25	24	26
4–6	22	20	16	19	16	16	17	16	18
7–9	20	18	14	18	14	14	16	15	16
10–12	20	19	15	18	13	14	15	14	16

TABLE 6

Nitrogen in the different sections

DEPTH	FIELD I	FIELD II	FIELD III	FIELD I	FIELD II	FIELD III
inches	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
	Canby			*Renville*		
1–3	0.39	0.40	0.38	0.50	0.42	0.42
4–6	0.29	0.29	0.25	0.35	0.36	0.31
7–9	0.23	0.23	0.18	0.27	0.33	0.26
10–12	0.19	0.18	0.13	0.19	0.28	0.21
	Hamel			*Taylors Falls*		
1–3	0.33	0.29	0.27	0.29	0.32	0.25
4–6	0.13	0.09	0.08	0.08	0.07	0.07
7–9	0.09	0.05	0.05	0.03	0.04	0.04
10–12	0.07	0.06	0.04	0.03	0.03	0.04

The values for Taylors Falls and Hamel are very similar but those for Canby are higher than those for Renville.

The soil of the prairie fields is much finer in texture as indicated by the moisture equivalents (table 5) but the magnitude of the moisture equivalent de-

pends upon the proportion of organic matter as well as upon the texture of the mineral portion. In the first foot the prairie fields are much the richer in organic matter, as indicated by the nitrogen determinations (table 6). Hence the evidence in hand does not permit the author to determine to what extent the lower density of the prairie soil is due to either the higher proportion of organic matter or the larger proportion of fine soil particles, or to the influence of differences in climate and plant cover.

Although the difference in the densities of the forest and prairie fields may be due to some extent to differences in texture, it probably is more largely due to differences in the content of organic matter. In the forest the organic matter is largely confined to the forest floor and the first two or three inches of soil under the leafmold, with a consequent lesser density of the 1 to 3-inch section, which may explain the sharp increase in density found in passing to the lower sections. In the prairie, on the other hand, where the organic matter content is largely derived from the grass roots, which penetrate for some distance below the surface foot, there is a higher content of organic matter and this is more uniformly distributed, resulting in a very gradual increase in density from the surface downward.

TABLE 7

Weight per acre of surface soil, computed for various depths*

DEPTH	PRAIRIE			FOREST		
	Canby	Renville	Average	Hamel	Taylors Falls	Average
inches	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
1–6⅔	1,551,000	1,359,000	1,445,000	1,664,000	1,709,000	1,687,000
1–7	1,640,000	1,431,000	1,536,000	1,778,000	1,814,000	1,796,000
1–8	1,912,000	1,655,000	1,784,000	2,110,000	2,144,000	2,127,000
1–9	2,188,000	1,887,000	2,038,000	2,476,000	2,440,000	2,458,000

* By curve interpolation.

In computing the amounts of plant nutrients per acre of land it is most customary to assume 2,000,000 pounds for the surface soil—the portion turned by the plow. This depth is most commonly placed at 6⅔ inches, as suggested by Hopkins (3, p. 59) although some place it at 7 and others at 8 inches. For constituents which vary little with depth, such as potash, the assumption of arbitrary depth and weight will not seriously affect comparisons, but with the nitrogen and organic matter it is quite different, as with these any depth of sampling other than that necessary to give 2,000,000 pounds per acre will cause considerable error. This depth varies, being 7.5 inches for the Hamel group, 7.7 for that at Taylors Falls, 8.3 inches for that at Canby, and 9.5 at Renville (table 7).

SUMMARY

The density of the soil *in situ* was determined in the four 3-inch sections of the surface foot of twelve Minnesota virgin fields developed on the till

plains of the Des Moines lobe of the Late Wisconsin drift. Six of these fields were in the deciduous forest and six on the open prairie. In the surface section the density of the soil was much alike in prairie and forest, but below that it increased gradually in the former but rapidly in the latter, being one-third the higher in the lower two sections of the forest soil.

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BOOK REVIEW[1]

Enzymes. By SELMAN A. WAKSMAN, Associate Professor of Soil Microbiology, Rutgers University; Microbiologist, New Jersey Agricultural Experiment Station; and WILBURT C. DAVISON, Associate Professor of Pediatrics, Johns Hopkins University, School of Medicine; Associate Pediatrician, Johns Hopkins Hospital. The Williams & Wilkins Company, Baltimore, 1926. Pp. xii + 350, bibliog., illus. 15 x 22.5 cm. Price $5.50.

The vast amount of material that has been accumulated in recent years as the result of studies in the field of enzymes makes the writing of a condensed presentation a difficult matter. The authors have succeeded admirably, however, in making the important results and laboratory methods available in a concise form.

The material has been arranged in four main sections. In section A, Properties of Enzymes, special attention is given to the physical chemistry of enzyme reactions and the factors which influence them. Enzymes of the human and animal body receive the greatest share of attention in section B, Distribution of Enzymes, and this part of the book should be of particular interest to workers in the medical field, to animal physiologists, and to biochemists. Almost one-half of the book is devoted to section C, Methods for the Preparation and Study of Enzymes. In this section, the enzymes are grouped according to the substrate on which they act, and methods of preparation, distribution, optimal conditions, and methods of measurement are given. The detailed directions of this section will be of value to laboratory workers in this field. As compared with the other divisions of the book, section D, Practical Applications of Enzyme Activity, receives only brief attention. The treatment is sufficient, however, to impress one with the extent to which enzyme reactions are applied in the industrial field, and suggestions are made as to possible future applications.

The book is replete with references to the literature, of which over thirteen hundred are listed at the end of the book, in a bibliography arranged alphabetically as to authors and also according to reference numbers in the text. This feature of the book will be of special value to research workers. The arrangement of the book is such that the material is easily accessible, the detailed outline of the table of contents appearing as paragraph headings. The book is thus very suitable for ready reference.

The conciseness of presentation has made the style somewhat abrupt in parts of the book, and for this reason it is not so readable as a more extensive

[1] This review was prepared by Prof. Walter C. Russell, of the New Jersey Agricultural Experiment Station.

work might be. Throughout, the emphasis is on facts and practise rather than on theory, and the treatment is too condensed to be of a critical nature.

The extensive bibliography, the brief theoretical treatment, and the wealth of laboratory methods should commend this book to workers who have to deal with enzyme reactions.